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THE TISSUE-INVASIVE POWERS OF THE FLAGELLATED AND CILIATED PROTOZOA WITH ESPECIAL REFERENCE TO TRICHOMONAS INTESTINALIS. A CRITICAL REVIEW*

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ONE TEXT FIGURE

The recent work of Hadley,(20) in which he has demonstrated that under certain conditions, so far unascertained, *Trichomonas* may become a destructive cytozoic and histozoic parasite, has again brought up the question of the tissue-invasive powers and pathogenicity of the flagellated and ciliated protozoan parasites found in the intestine of man. The question is of such prime importance in the practice of medicine, particularly in the tropics, that it seems opportune at this time to review some of the literature on these very important subjects. It must be confessed at the beginning that it seems impossible to draw any very definite conclusions; but force is added to the already very prevalent impression that the flagellated intestinal protozoa should be viewed with suspicion and regarded as pathogenic until the contrary is proved beyond dispute.

From the viewpoint of human pathology the matter can be scarcely discussed at this time, for with the exception of *Balantidium* infections of the human intestinal tract there are no observations upon which to work. The whole problem from the clinical, pathological, and experimental viewpoints presents ex-

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ceptional difficulties, both to the pathologist and to the protozoölogist.

A review of the situation brings out some points in parasitology of great interest from both the theoretical and practical viewpoints and raises some questions that promise to form the basis for an interesting series of studies. Among other questions raised is that that bears on the effects produced upon a parasite by the internal reactions of the host, as expressed through the tissues and body fluids. In this connection I mean reactions of the host that tend to favor the parasite as distinguished from the familiar reactions that work to the disadvantage of the parasite. In the past the attention of parasitologists and physicians has been focused largely upon the effects of the parasite upon its host—a most natural point of view. But in reviewing the subject, especially in the light of recent evidence, it is hard to escape the impression that the host, on occasion, may transform an apparently harmless parasite into one that is pathogenic or even lethal to its host. This is entirely apart from conditions of lowered vitality and resistance, which proverbially favor the development of infections of all kinds. It would seem that the term "harmless commensal" has been very much overworked. The case of the intestinal nematode *Ascaris lumbricoides* is very much in point, and apparently the day is not far distant when the terms "harmless commensal" and "symbiont" will be used in the literature with much greater caution than has been shown in the past. It has long been my belief that time would show that all animal parasites that have been regarded as commensals and symbionts in the alimentary tract, if not actually giving rise to lesions, would yet be shown to affect unfavorably the physiological balance of the host in some way.

Recently this has been strikingly indicated by Gibson,(17) who has noted the apparent restraining influence of *Ascaris* infection on the growth of puppies. The phenomena were, to be sure, observed in the course of only one experiment, and certain other factors may have intervened to bring about the results he cites, but they certainly are suggestive. Gibson says:

> The existence of an anti-vitamine or at least of growth inhibiting substances formed by ascarids is suggested by an observation which I made in connection with some milk feeding experiments with puppies. In a series of five young puppies fed on cows' milk growth stopped in four of the animals when 44 days old. Following the administration of an efficient vermifuge, there resulted the passage of many ascarids from the four dogs in which growth had ceased. Growth was immediately reëstablished.

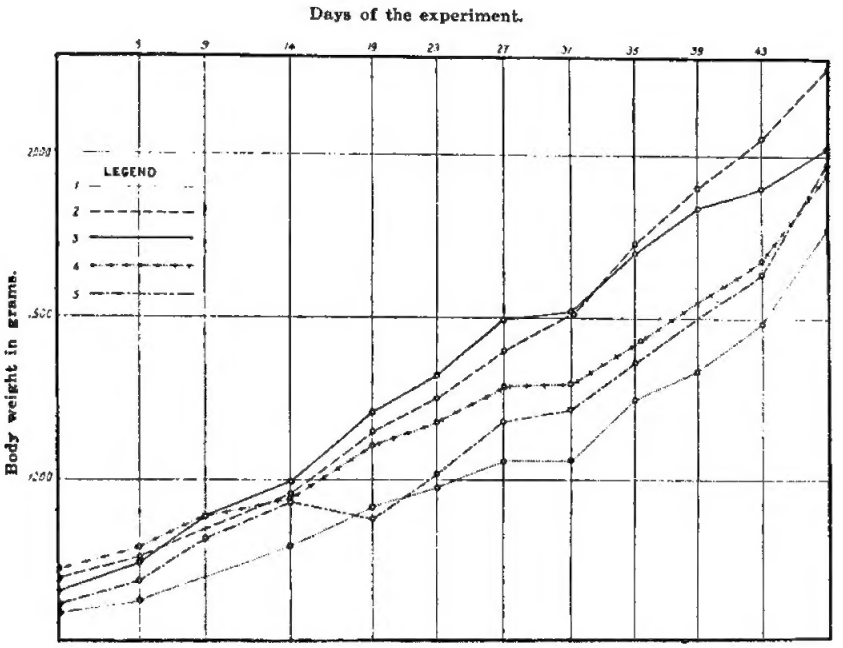

FIG. 1. Growth curves for dogs fed on fresh and autoclaved milk. Note the falling off of growth between the twenty-seventh and the thirty-first day. Note also that dog 2, which was not infected with *Ascaris*, continued to grow at about the normal rate.

The growth chart illustrating this case has been published by Gibson and Concepcion (18) and is reproduced here as fig. 1, by kind permission of the authors.

This is suggestive of the pernicious effects that may occur as a result of heavy infections of protozoa of the so-called "harmless commensal" type, particularly in very young children, and is entirely apart from the menace they afford as possible tissue invaders. It would seem that the end products of protozoan metabolism, in cases where a host is heavily infected, could not fail to affect the host unfavorably, particularly in the upper intestine, where absorption is active. It is known that protozoa in cultures may themselves succumb to the products of their own metabolism, as has been shown by Woodruff (53) in a series of experiments with the free-living infusorian *Paramœcium*. In connection with the suggestion advanced by Gibson, it should be borne in mind that the effects he noted may have simply been those of starvation, through the appropriation by the parasite of food designed for the host, an effect which would probably be particularly noticeable in the case of a young and growing animal.

It has occurred to me that the condition I have mentioned might possibly explain some of the frequent and obscure cases of "auto-intoxication" occurring in the tropics, often under apparently unexplainable conditions; and possibly, also, the case presented by the patient in the tropics who is unable to give his physician any further information than that he experiences a disinclination to work and "feels rotten"—a condition often referred to as "Philippinitis," in the Philippine Islands. I have several times been struck by the fact that a large proportion of such individuals harbored swarms of trichomonads, when there was not a frank infection with helminths, and I have been inclined to suspect that had the opportunity been present for an examination of the stools on several successive days the flagellates would have appeared in the other cases as well. I have noted similar symptoms in individuals suffering from heavy infections with *Spirochæta eurygyrata.*

Trichomonas is a protozoan parasite of the order Polymastigida and the subphylum Mastigophora. It is found through a wide range of the lower animals and very frequently in man. It possesses well-developed motile organs and a cytostome for the ingestion of solid food and is, in no sense, to be regarded as a primitive or degenerate species. Its nucleus is of the karyosome type and divides with a well-developed mitotic figure in which chromosomes have been demonstrated in some species. Its life cycle, so far as it has been worked out, seems to be fairly complex. Although the behavior of the chromatin under certain conditions may be regarded as leading up to it, syngamy has not yet been worked out.

The organism shows more or less variation in size and form. *Trichomonas intestinalis* of man measures, generally speaking, from 10 to 15 μ in length and from 3 to 5 μ at its greatest breadth. Forms may be found that are almost spherical through those that are triangular, short, or elongated. Many are pyriform or pear-shaped. The organisms are active in their movements, but frequently come to rest, apparently to feed. Their food during life in the lumen of the intestine consists, for the most part, of bacteria. Three equal flagella, inserted in relation to a basal granule or blepharoplast, spring from the anterior end of the body and are employed in locomotion and food-taking. Another, somewhat stouter flagellum runs posteriorly, forming the margin of an extension of the ectoplasm, the undulating membrane, and is continued as a free lash beyond the posterior end. The inner margin of the undulating membrane is bordered by a deeply staining linear struc-

ture, the parabasal body. A clear, refractile, rodlike structure, originating at about the middle third of the body, runs posteriorly and frequently projects beyond the posterior extremity of the body. This is the axostyle. By some authors this is believed to be a stiffening rod for maintaining the contour of the body. Others speak of it as an attaching organ, while Kofoid and Swezy(26) state that it is in the nature of a motile organ, or, as they say, "a stout, largely intracytoplasmic flagellum for locomotion in a viscid medium." It has occurred to me that it may, under some circumstances, serve as a piercing organ in connection with tissue penetration. The cytostome is a triangular depression at the anterior end, laterad of the median line. Some writers describe a flagellum within it, an observation I am unable to confirm. The endoplasm contains one or more vacuoles, apparently food vacuoles, for bacteria may be frequently seen within them. I have never observed a contractile vacuole. Excretion may take place by diffusion through the body wall or through the cytostome.

Trichomonas is frequently found to bear four or even five anterior flagella, which has caused certain writers to place them in different genera under the euphonious, but somewhat anomalous names *Tetratrichomonas* and *Pentatrichomonas*. I shall not take the time here to discuss the advisability or inadvisability of founding new genera on such trivial variations as these.

Ohira and Noguchi(40) have created five subgenera to be included under the genus *Trichomonas* Donné, 1837. They are:

1. *Protrichomonas* Alexeieff, with three anterior flagella, without an undulating membrane.
2. *Trichomastix* Bütschli, with three anterior flagella and a trailing flagellum (*Schleppgeissel*), without an undulating membrane.
3. *Trichomonas* Donné, with three anterior flagella and an undulating membrane.
4. *Macrostoma* Alexeieff, emend. Wenyon, with three anterior flagella, and an undulating membrane wedged in a deep groove (peristome).
5. *Tetratrichomonas* Parisi, with four anterior flagella and an undulating membrane.

To these might be added Chatterjee's *Pentatrichomonas*, with five anterior flagella and an undulating membrane. Other intestinal flagellates of interest here are:

Lamblia (*Giardia*) *intestinalis* Lambl, 1859. This is an organism of peculiarly striking and characteristic appearance and

one of the few protozoa that are bilaterally symmetrical. The organism is pear-shaped and measures from 10 to 21 μ, with a width of from 5 to 12 μ. There is an anterior, oblique depression or sucking disk, the contractile edges of which are raised above the general surface. There is no mouth or cytostome. Two axostyles extend from the region of the sucking disk to the posterior end, where they continue as two stout flagella. There are three other pairs of flagella arising from the borders of the hollow disk, giving eight flagella in all. The organism, as usually seen, is binucleate, the two nuclei of the karyosome type lying within the sucking disk, one on each side of the axostyles. Stained, the organism bears a most grotesque resemblance to a spectacled face. This resemblance extends even to the cysts.

Enteromonas hominis da Fonseca, 1915. This organism has been placed in the Tetramitidinæ by Chalmers and Pekkola.[2] They describe it as being equipped with three unequal anterior flagella, lacking a permanent cytostome, undulating membrane, and axostyle. The nucleus is of the protokaryon type and is joined to the blepharoplasts by a rhizoplast. This organism they believe to be allied to the genus *Dallengeria* Saville-Kent, 1880. Chalmers and Pekkola regard this organism as pathogenic to man, giving rise to diarrhœa, "and by absorption from the bowel febrile attacks." They record three cases of this infection, the first being reported from Brazil by da Fonseca and the other two in the Anglo-Egyptian Sudan by themselves. They venture the prediction that it may be ultimately found to be widespread throughout the tropics.

Tetramitus mesnili Wenyon, 1910. See *Macrostoma*.

Cercomonas hominis Davaine, 1854. *Cercomonas* is a very uncertain genus, but a great convenience to those who are unable otherwise to classify an intestinal flagellate. The descriptions vary greatly, and undoubtedly other flagellates such as *Trichomonas*, *Macrostoma*, and the like have been called *Cercomonas* by unskilled observers. *Cercomonas* is described as having a pear-shaped body, 10 to 12 μ long, which tapers to a point posteriorly. The flagellum is said to be about 20 μ long. A cytostome has been described by some writers.

Prowazekia Hartmann and Chagas, 1910. Species reported as occurring in fæces are: *Prowazekia cruzi*, *Prowazekia asiatica*, *Prowazekia weinbergi*, and *Prowazekia javanensis*. The general characteristics of this genus are the possession of two flagella,

[2] *Bull. Soc. path. exot.* (1917), 10, 756.

one of which is directed forward and the other downward and backward in heteromastigote fashion. There is a principal nucleus (trophonucleus ?), and there is another body of supposed nuclear nature that has been variously termed the kinetonucleus, or the blepharoplast. Absolute proof of the parasitic nature of this organism is still lacking, and there is a tendency to regard it as a free-living form, which has contaminated stools or urine. I have never seen it in pond or tap water, but some observers have reported it as occurring free.[3]

To my mind there is some ground for suspecting that *Protrichomonas, Trichomastix, Macrostoma,* and possibly *Cercomonas* may represent developmental stages in the life cycle of *Trichomonas,* and Minchin(35) suggests that as *Trichomonas* and *Trichomastix* frequently occur in the same host they are "perhaps to be interpreted as two developmental phases of the same organism rather than as distinct generic types."

Reproduction in *Trichomonas* has been described by several writers as taking the form of simple longitudinal binary fission, which may, at times, give way to multiple fission. Recent investigations seem to indicate that either form of reproduction may occur. Cyst formation is unproved, many writers doubting if it occurs.

Woodcock(52) considers that *Trichomonas* may have lost the power to produce cysts and considers it likely that "*infection with trichomonas can take place by means of the active, unencysted forms.*" (The italics are Woodcock's.) He found that *Trichomonas* would live and remain active for five and one-half hours, both in 0.066 hydrochloric acid solution and also in pancreated solution of a strength that would bring about the excystation of *Entamœba histolytica.*

In this connection Wenyon(50) states that the rounded-out forms are capable of resisting the action of gastric juice for a considerable time, which might, as Woodcock says, account for the safe passage of the naked parasites through the stomach. The bodies appearing in the fæces of man and of some of the lower animals and generally spoken of as *Blastocystis enterocola* or *Blastocystis hominis* have been thought by many to be

[3] Macfie (*Journ. Trop. Med. & Hyg.* (1917), 20, 1), reporting from Accra, states that flagellates soon make their appearance in bottles of saline solution or distilled water exposed in the laboratory. Apparently the organisms develop in the thin layer of fluid between the neck of the bottle and the ground glass stopper. They are not usually found in the fluids inside the bottles. These flagellates appeared to belong to the genus *Prowazekia* of Hartmann and Chagas.

the cysts of *Trichomonas*, but as will be shown this remains to be proved. Prowazek,(43) following a study of them, has declared his belief that they are the cysts of a flagellate, *Bodo lacertæ*. Added evidence of their flagellate nature has been recently produced by Chatton,(9) and further presumptive evidence is gained by a study of the plate illustrating Hadley's paper.(20)

A review of some of the clinical observations on flagellate infections discloses a variety of opinions, which coincide somewhat with the views that have been held regarding the etiology of *Entamœba histolytica* in dysentery. Some writers frankly state their belief that *Trichomonas* and some of the other flagellates are pathogenic and that they may give rise to the symptom complex known as dysentery. Others believe that they will produce nothing worse than diarrhœa, while still others adopt an intermediate position in holding that while incapable of initiating lesions they may aggravate preëxisting lesions. Brumpt(4) has described a case of colitis in a patient returned to France from Tonkin that he ascribes to infection with *Trichomonas intestinalis*. Escomel(11) has written of one hundred fifty-two cases he speaks of as dysentery, occurring in Peru. The causative organism he believes to have been *Trichomonas* and claims that the source of infection was traced to a polluted water supply. Mello-Leitao(31) traced cases of dysentery in children in Rio de Janeiro to *Trichomonas intestinalis* and *Lamblia intestinalis*, occurring either separately or together. He believes that flagellate dysentery is benign, but that the organisms may be pathogenic to children under 3 years of age. This form of dysentery, he thinks, is the most frequent type occurring in infants. Derrieu and Raynaud(10) report a case of chronic dysentery in Algiers, which they lay at the door of *Pentatrichomonas bengalensis* Chatterjee, 1915. In the United States, Rhamy and Metts(44) state their strong belief in the pathogenicity of *Trichomonas intestinalis* and give detailed clinical reports of several cases. The senior author says that in an experience extending over seventeen years he has never found flagellated protozoa in stools except in cases with existing or recent acute or chronic diarrhœa. He states his belief that sufficient importance has not been placed on the pathogenicity of this parasite.

Rhamy and Metts review an epidemic of flagellate dysentery involving seventy-eight cases with seventeen deaths and they also present seven selected cases of their own, none of which, however, went to autopsy. These cases they characterize as "dysenteric diarrhœa." They state that so far as they could

ascertain the disturbances were caused by the flagellated protozoa. They go on to say, however, that cases recovered under antidysenteric treatment with ipecac and its compounds. This appears to be at variance with the experience of other workers, who almost unanimously report unsatisfactory results in the treatment of flagellate infections with ipecac and raises the question of the possibility that the authors might have overlooked *Entamœba histolytica* in their examination of the stools. Apparently bacillary dysentery was not absolutely ruled out. The cases they trace to impure water.

These authors describe the symptoms as consisting of diarrhœa with colicky pains, watery or slimy blood-stained stools, weakness, dyspnœa, loss of weight, progressive anæmia simulating pernicious anæmia, and the skin appearing yellow with urticarial or pellagroid eruptions. Later the stools consisted of mucus, blood, pus, and active trichomonads. The blood showed a moderate eosinophilia (6 to 12 per cent). Proctoscopic examination of one of their cases showed the presence of a large shallow ulcer on the posterior wall of the rectum. The remainder of the rectum was covered with mucus and was congested. The stools of this patient contained blood, pus, and trichomonads.

A complete description is not given of the organisms seen by these authors; that is to say, the description is not sufficiently complete to leave their identity as *Trichomonas* beyond question, and indeed, such is the case with many of the reports that are available to me. This makes it difficult in some instances to decide what flagellate is involved.

Of interest in connection with this observation is a case I recently saw in consultation with Dr. A. F. Coutant, at St. Luke's Hospital, Manila. The patient was a young American woman, married, who some time previous had suffered an acute attack of intestinal entamœbiasis. She came to the hospital for treatment of a persistent diarrhœa, which, though troublesome, did not prevent her from going about. Repeated examination of the stools of this patient failed to disclose the presence of entamœbæ, but the stools contained considerable mucus and an occasional red blood corpuscle. Eventually *Trichomonas intestinalis* was found in small numbers. Proctoscopic examination of the patient disclosed a small eroded area on the wall of the rectum. This spot was not ulcerated. A small mass of mucus was carefully removed from this eroded area with a sterile platinum loop. Microscopic examination of this mucus revealed a large number of active trichomonads. Under treatment with

methylene blue the trichomonads disappeared and with them the diarrhœa. Soon afterward the patient left for the United States, and the case passed out of observation.

Prentiss(42) reports several cases of infection with an organism he describes as *Cercomonas hominis*. He describes it as a "flagellated protozoön, bluntly pointed at both extremities, with a thin flagellum at one end, which by waving actively imparts motion to the organism similar to that of a tadpole." This organism he has found in the fæces of a number of his patients in the southwestern United States (he writes from El Paso, Texas). This parasite was found in the fæces of patients having diarrhœa—some chronic, others acute. *Entamœba histolytica* was not found. Some of the cases assumed a severe form, and two terminated fatally with symptoms suggestive more of an acute catarrh than of ulceration. In one fatal case that was necropsied, the intestine showed no trace of ulceration. The author says no other cause of death could be found. The identity of the organism seems to be in doubt here.

Chatterjee,(8) however, is more explicit. He boldly designates flagellate dysentery as "a distinct entity." He studied seventy cases in India and tabulates his results in Table I as follows:

TABLE I.

Organism.	Character of stool.	Number of cases observed.
Monocercomonas	Choleraic	3
Prowazekia	do	2
Macrostoma	Chronic dysentery	18
Lamblia	do	15
Pentatrichomonas	do	32

He concludes that *Macrostoma*, *Lamblia*, and *Pentatrichomonas* cause intestinal trouble. *Monocercomonas* and *Prowazekia* he regards as harmless, pointing out their occurrence in cholera motions. To this latter statement I may add, as I have already said, that the systematic position of the cercomonads is uncertain,[4] and *Prowazekia* is thought by many to occur as a contamination of fæces or urine and not to be a parasite.

Nearly every writer on tropical medicine has spoken of diarrhœa as a concomitant of flagellate infection. Musgrave(38)

[4] *Monocercomonas*, according to Alexeieff, has four anterior flagella. Sometimes these are of equal length, or two may be shorter and two longer.

has given voice to the belief of the early workers in Manila that many of these so-called harmless parasites are disease producers. He adds that several of the Manila men (he wrote in 1906) recognized a diarrhœa caused by "monads." He says:

> Several types of these parasites, when present in large numbers, are very intimately connected with chronic diarrhœa and they are surely much more important than they are generally considered to be. [p. 561.]

He views *Lamblia intestinalis* with particular suspicion and goes on to say that "when encountered in great numbers, it is *always* associated with chronic diarrhœa, which disappears with the destruction of the parasites."

With *Lamblia* the conditions to me seem to be more obvious, for here we have a parasite that we know attaches itself to the epithelium by its sucking disk. It seems reasonable to conclude that this alone would give rise to considerable irritation, to say nothing of the facility with which toxic excretory products of the parasite could be absorbed by the epithelium with which it is in such close relation. In the cases of the other flagellated protozoa we have less information on that point. Musgrave's belief is that *Lamblia* "bears a decided causative relation to the diarrhœa."

Speaking of the intestinal parasites in general, including the helminths, Musgrave concludes that—

> Whatever pathological significance may be attached to these parasites in general, some of them, particularly the actively motile ones, such as monads, surely aggravate amœbic ulcers in which they may be present.

Fantham, Stephens, and Theobald(14) say that "like *Trichomonas, Lamblia* can multiply under inflammatory conditions of the alimentary tract."

Fantham and Porter,(12) following a series of carefully conducted experiments, reached the conclusion that *Lamblia* is pathogenic to man and is capable of producing diarrhœa, which may be persistent or recurrent. Furthermore they hold the view, which to me is of great importance in considering the relations of the intestinal flagellates to man, that the virulence of the parasite varies and lambliasis occurs in tropical and nontropical countries. *Lamblia* cysts, they say, will remain infective for some time.

Whatever the case, the flagellates certainly often persist in the stools for long periods of time, and diarrhœa is the rule when they are present in large numbers. Whether they cause the diarrhœa or whether the increase in their number is brought about by the diarrhœa is quite another question. The most

charitable attitude from the viewpoint of *Trichomonas* is taken by Minchin(33) who says:

> The common intestinal flagellates belonging to the genus *Trichomonas* and other genera are * * * not to be regarded as true parasites in any sense of the word * * * Many of these intestinal Protozoa are perhaps useful, rather than harmful to their host.

At least four authors report infection with *Trichomonas* through the drinking of impure water, and in at least two cases there were epidemic outbreaks alleged to be due to *Trichomonas*. Smithies(47) reports two cases of severe dyspepsia in which he recovered *Trichomonas* from the stomach. These cases occurred in the southern United States. The infection in one case he attributes to the drinking of unfiltered surface water by the patient. In the epidemic in Peru, reported by Escomel,(11) which I have already mentioned, he states that examination of the reservoirs containing the water used for drinking purposes showed the presence of *Trichomonas*. When the reservoirs were cleaned, the organisms disappeared, and the outbreak ceased. As will be shown later on, *Trichomonas* has been cultivated by at least two groups of workers, and it is a fact well known to all tropical workers that the parasites will survive in stools for many days. Kofoid and Swezy(26) made cover glass preparations of *Trichomonas augusta*, which they diluted with normal salt solution and sealed with vaseline. They report that the parasites were "kept alive for several months without any change in the medium, or removal of the cover glass." I have had the same experience with *Trichomonas lacertæ*, which I have kept in mounts sealed with paraffin, the organisms continuing to live in physiological salt solution for upward of six weeks. At the end of that time the sealing of the mounts became loosened, and the preparations dried with consequent death of the organisms. Lastly there is the epidemic of trichomoniasis reported by Rhamy and Metts,(44) as involving seventy-eight patients at Liberty Township, Indiana, in 1909.

Of course, in all these cases possible mistakes in the identity of the forms mentioned must be borne in mind. While undulating membranes of the type seen in *Trypanosoma* and *Trichomonas* are characteristic of the parasitic species, still there remains the possibility of mistaking some of the multiflagellate species of free-living protozoa for *Trichomonas* and other intestinal flagellates.

I might, in passing, remark that I have received, on more than one occasion, contaminated stools or urine that have contained undoubted fresh-water forms such as *Phacus*, *Arcella*, and *Pera-*

nema, and have been asked to identify these new intestinal or vesical parasites. Unfamiliarity with free-living species has undoubtedly led to the discovery of "new protozoan parasites" on some occasions. This should not, however, be construed either as a reflection on the powers of observation of these authors or a denial of the possibility that *Trichomonas* may be capable of living in pond water. Like *Balantidium*, its food-getting apparatus is adequate, and it is likely that it could secure its proper food in sewage-polluted waters. But it remains to demonstrate the fact experimentally one way or the other or by the observation of the organisms in pond water.

There was an outbreak of diarrhœa at Parañaque, a suburb of Manila, in 1914. The local sanitary inspector at that place assured me at the time that the diarrhœa was epidemic in the town and that there had been a large number of cases, some of which had been attended with fatal results. He stated his belief that the trouble was due to *Trichomonas*, with which he thought the sufferers had been infected through drinking river water. He had no material at hand that I could study, and pressure of other duties made it impossible for me to investigate the matter further.

Fantham, Stephens, and Theobald(13) believe that air, water, and on some occasions food may be vectors of *Trichomonas*.

As for *Lamblia*, Mathis(30) and Fantham and Porter(12) say rats are transmitters and reservoirs for the species infesting man. A similar condition may exist in the case of *Trichomonas* and the other intestinal flagellates. It is pretty well established that pigs are reservoirs and transmitters of *Balantidium coli*, and the rat is under suspicion in connection with entamœbiasis.

Unfortunately Hadley(20) does not figure the intracellular stages of *Trichomonas* in his cases of blackhead in turkeys, and we shall have to await his future communications for these. It would be interesting to see how closely his intercellular parasites coincide in appearance with *Entamœba histolytica* as the latter appear in sections through the intestine. Shorn of its flagella, undulating membrane, and axostyle and possessing a nucleus of the karyosome type characteristic of the entamœbæ, it is not hard to see how *Trichomonas* could have been mistaken for *Entamœba* even by careful workers.

Hadley speaks of the trophozoite stage in the lumen of the intestine, as the period of youth during which the organism divides by simple, longitudinal fission and accumulates a reserve of food substance in the endoplasm. When this food reserve has accumulated to a sufficient amount, this method of reproduc-

tion gives way to what Hadley styles a form of "autogamous reproduction." This he has not worked out in all its details, and I am unable to determine if it is autogamy or merely multiple division within a cyst, although he mentions the casting off of nuclear material, which might be interpreted as a process of nuclear reduction. The fusion of gametic nuclei, such as has been described by Wenyon (51) for *Entamœba muris*, is not mentioned by Hadley, but he speaks of the possible conjugation (copulation?) of flagellates derived from the original trophozoite. These flagellates might be in the nature of swarmers produced after the reduction process spoken of by Hadley, or they might be trophozoites undergoing copulation as has been described by Dobell in the case of *Copromonas subtilis*. Schaudinn in short note, (45) has stated that *Trichomonas* becomes an amœba and that two of these amœbæ, after giving off reduction nuclei, encyst together and carry out syngamy within the cyst. Later the zygote breaks up, forming small individuals, leaving a mass of residual protoplasm behind. However, this has not been confirmed, and indeed the whole process of syngamy in *Trichomonas* remains to be worked out, as does the same problem in the larger number of intestinal protozoan parasites.

In the process described by Hadley, the trophozoite usually increases in size, rounds off, and secretes a cyst. The parabasal body lengthens, until it forms almost a complete circle near the periphery. The flagella, undulating membrane, and cytostome are gradually lost, and the vacuole or vacuoles of the trophozoite coalesce and enlarge until the single vacuole occupies the greater portion of the ventral part of the animal. The cytoplasm and nucleus are flattened against the dorsal wall. The chromatinic blocks and axostyle degenerate and disappear. The parabasal may persist with the blepharoplast for a time, but eventually they, too, disappear.

At this stage the vacuole, which is held to contain the store of reserve food, has increased in size, until it occupies the larger part of the cell and is surrounded by a crescentic ring of cytoplasm, which seemingly has become much reduced in amount. The nucleus is flattened or flask-shaped and divides, the daughter nuclei taking up positions at opposite sides of the food vacuole. At this stage the organism certainly bears a striking resemblance to *Blastocystis* and to the figures in Chatton's recent paper. The nuclei may then divide to form four, eight, or sixteen daughter nuclei, which arrange themselves about the periphery.

Hadley mentions at this point that smaller portions of nuclear

substance may be seen in the cytoplasm following the first nuclear division and states his belief that they represent the reduction bodies I have already mentioned. He has not observed them in the process of formation.

Following the multiple division of the nucleus, cytoplasm collects around each nucleus, and the small cells thus formed break from the peripheral ring and enter the centrally situated food vacuole, which would then appear to become something in the nature of a brood chamber. The substance within this vacuole seems to be gradually consumed by the cells, which grow in size. Next the cyst wall weakens, the young organisms burst out and swim off as trophozoites, measuring 4 to 5 μ in length by 3 μ in width, each equipped with an anterior flagellum, a nucleus, and a blepharoplast. Gradually these young forms develop additional flagella and the other organelles characteristic of *Trichomonas*. During this series of developmental changes it would appear that the organism might come to resemble Wenyon's *Macrostoma mesnili*.

It is at this stage that Hadley says he has evidence of conjugation between two or three or even four individuals. This conjugation of more than two individuals would constitute something of a departure from the process usually observed in the Protozoa where syngamy occurs (when it is not autogamous) between two individuals only. It is hard not to regard the union of three or even four individuals under such circumstances as being wholly fortuitous and probably unproductive of results. At periods of sexual maturity in the Protozoa it is not infrequently observed that the ectoplasm of the organisms becomes sticky, so that they have a tendency to adhere in pairs if they blunder against each other. This is strikingly seen in the conjugation of *Paramœcium caudatum;* and it is not very unusual when epidemics of conjugation occur in a culture, as they frequently do, to see three or four irregularly attached individuals swimming about in a clump. Usually the matter adjusts itself with the aid of the ciliary currents of the animals, which tend to bring them into the proper position for carrying out the process of conjugation.

If conjugation or copulation of these flagellated individuals takes place, as Hadley suspects may be the case, it seems to be a process of either endogamy or exogamy, depending, of course, upon whether union took place between cells derived from the same or from different cysts. In that case Hadley's autogamy would fall and the process of nuclear reduction he suggests

might be, as I have already said, a reduction process preliminary to the formation of flagellated gametes.[5]

Following the copulation of these flagellated cells, Hadley mentions the formation of a viscid membrane around the organisms, which have meanwhile rounded off. This might coincide with the process described by Schaudinn (45) mentioned previously. Following conjugation, adds Hadley, the membrane hardens to form a firm cyst. The single cysts, he says, measure from 10 to 12 μ, but in the "fused" or conjugated forms the cysts may reach a size of 20 to 30 μ. "Double" or "triple" cysts, he concludes, may represent a division of the original cysts, whereupon each cyst continues to produce daughter cells independently.

This stage of the life cycle, according to Hadley, is the one usually encountered and which has caused *Trichomonas* to be regarded as a harmless commensal; but he goes on to show that in another phase of activity the parasite may penetrate the epithelium and cause fatal lesions in the intestinal tract. The question is, what stimulus is it that causes the organism so radically to alter its mode of life. To my mind this very question is to-day one of the most important problems of parasitology.

In the turkeys observed by Hadley the invasion was invariably preceded by diarrhœa with an accompanying increase in the number of parasites as the disease advanced. The parasites appear not only in the liquid cæcal contents, but in the depths of the cæcal tubules or crypts and finally in the tissues behind the epithelial walls. Hadley states that by a process of "autogamous reproduction" the mucosa, submucosa, and muscularis mucosæ and even the muscular layers are successively invaded, until the whole cæcal wall is involved. Secondary bacterial invasions may supervene to bring about results that are almost invariably fatal. Here Hadley raises the old question as to whether the vast numbers of parasites present are the cause or the result of the diarrhœa. This point will be further discussed.

[5] In a recent letter to me, Doctor Hadley says: "Regarding the presence of syngamy in the reproductive process, I doubt very much that it occurs in the flagellated swarmers. I have never seen the least suggestion of it. I am rather of the opinion that, when it occurs at all, it takes place in the stage after the organism has lost its chief features of flagellate morphology, has become globoid and possesses a more or less viscid capsule or membrane. In fresh preparations such appearances, at least in the first adhesive stages, are fairly common." It is a matter of great regret to me that three other papers by Doctor Hadley, dealing with this subject, reached me too late for consideration in connection with this paper. They should be consulted by all who are interested in the subject. [See *Bull. Agr. Exp. Station*, Rhode Island State College, Kingston, R. I. (1916), Nos. 166 and 168, and *Journ. Med. Res.* (1917), 36, 79.]

Apparently the parasites gain entrance to the tissues through the goblet cells of the crypts of Lieberkühn. They accumulate in large numbers in the fundi of the crypts, causing the walls to bulge, with the result that the parasites are literally forced into the goblet cells, past the nucleus and cell wall, until finally they enter beneath the epithelium of the crypt. The passage once made, other trichomonads follow and collect in a mass between the epithelium and the basement membrane.

This tendency to collect about the surface of the epithelium has been noted by other workers. Kofoid and Swezy(26) and Martin and Robertson(29) mention it in connection with their search for dividing forms.

Next the parasites push through the basement membrane into the connective tissue of the mucosa and thence to the muscularis mucosæ and submucosa. The invasion is described as being of an intracellular nature as in the case of the Sporozoa.

Smith(46) has discussed at length some peculiar bodies he found in 1916 in a turkey suffering supposedly from blackhead, which he was inclined at the time to regard as coccidia that had wandered beyond their accustomed habitat, the epithelium. Characteristic lesions of blackhead were absent, and several coccidial cysts were found in the fæces. The article is too long to quote in extenso, but the author mentions certain things that may have a bearing on Hadley's observations. Sections of the intestinal tract showed that the epithelium had been lifted from the core of the villus, leaving the space intervening filled with a precipitate of fine granules. The parasites appeared as an almost continuous band near the margin of the villus core. The striking feature seems to be the appearance of these bodies, which were vacuolelike and partially empty. A few were filled. Smith says:

> They consisted of some host cell whose cytoplasm had been moulded into a shell (or ring in section) with the much flattened nucleus against this shell. The contents were a very fine lining membrane within which were roundish bodies of various diameters 2 μ and more, staining feebly reddish and with or without a mass of chromatin. Frequently a body contained two chromatin masses situated at opposite poles, as if division had taken place. Those bodies which were full of spheres, contained about sixteen or more of more or less uniform size. The vacuolated appearance under low power was due to the disappearance of some or all of the parasitic contents of the host cell. Prolonged search for the characteristic products of asexual multiplication—falciform bodies—brought to light only two or three parasites containing them. It is not to be denied that these may have been moulded into crescent shape by the pressure of the other growing and segmenting members in the same membrane.

Were these bodies trichomonads undergoing Hadley's autogamous reproduction in the tissues? Undoubtedly the turkey that formed the material for Smith's study was infected with *Coccidium*, and parasites found in the epithelial cells were, according to Smith, clearly coccidia. It was the collection of bodies in the subepithelial tissues that was regarded by him as representing something different. However, he considered them to be aberrant coccidia, and the possibility of their being trichomonads is not discussed by him.

Going back to Hadley's account of *Trichomonas*, we note that this author mentions the engulfing of the flagellates by endothelial and other phagocytic cells. This, in many cases, seems to have had no untoward effect upon the parasites, which showed a marked resistance to the phagocytes and even seemed to divide within them. Instances in which infection with microörganisms is spread by phagocytes are known. *Hepatozoön perniciosum*, a sporozoan parasite of the rat, has been described by Miller(32) as passing a portion of its life cycle in encysted form in the large lymphocytes of the rat, and protozoan parasites of leucocytes have been long known. Hadley believes that the protozoa in his case use the host phagocyte as food and that they may, indeed, fare better within than without the phagocytic cells. In the regions of the invaded tissue he has found the majority of the parasites present in phagocytic cells, which he cites as proof of the rôle the phagocytes play in spreading the infection.

In the lumen of the intestine the trichomonads are holozoic forms, subsisting largely on the bacteria found there. In the tissues their morphology is markedly altered, and the evidence, according to Hadley, indicates the substitution of an osmotic method of nutrition. A point is raised here as to whether or not all tissue-dwelling forms are nourished by absorption or whether such forms as *Entamœba* and *Balantidium* do not continue to nourish themselves by the holozoic method. If, in the tissues, *Trichomonas* becomes virtually an amœba, does it take in solid food or does it derive its support from fluid substance absorbed through the body wall?

Hadley states that although the motile forms can be recognized in the tissues, as can some of the sporulating forms, these are relatively scarce, and the stage that shows the well-rounded ball of reserve food substance, which appears to be in the nature of glycogen, is seldom encountered. Further details as to the appearance of these parasites in the tissues are necessary before any final conclusions can be drawn regarding the presence of an altered form of *Trichomonas* in the human intestine.

The author (Hadley) points out the obvious difficulty of escape from the hosts that these parasites buried deep in the tissues would meet; but again we may find instances of this very contingency in the literature on the Protozoa, where the death of the host is necessary before the spores can be disseminated. However, he shows how the parasites may return to the lumen of the cæcum by spreading downward and inward through the cores of the villi, finally breaking through the epithelium by sheer pressure of numbers, much as they made their entrance.

Kofoid and Swezy,(26) in their studies of mitosis and multiple fission in the trichomonads, bring out several points that are of interest in connection with the work of Hadley. They state their inability to present conclusive evidence "that either leads to gamete formation by maturation divisions, or that either follows zygote formation or fertilization." This knowledge they believe will come only with the solution of the history of the "true trichomonad cysts."

There is a great diversity of opinion as to whether the encysted stage of *Trichomonas* or some other flagellate is represented by *Blastocystis*. Wenyon,(50) while admitting that on occasions it has seemed to him that degenerating *Trichomonas* or *Tetramitus* may become centrally vacuolated and resemble *Blastocystis*, thinks it is untenable to view the latter as encysted *Trichomonas*. Kofoid and Swezy are skeptical of the relation of *Blastocystis* to *Trichomonas* and figure *Trichomonas* with an engulfed cyst of *Blastocystis enterocola*. However, that does not necessarily constitute a conclusive argument, for I have seen *Vahlkampfia* engulf cysts of its own species. Galli-Valerio(15) found double-contoured cysts in the fæces of guinea pigs infected with *Trichomonas*, after the fæces had been kept in a damp chamber for one month. When warmed, the cysts opened and discharged small flagellates. Administration of the cysts caused infections in other animals. Alexeieff(1) and Brumpt(4) doubt encystment in *Trichomonas* and state their belief that these cysts (*Blastocystis*) represent a stage in the life cycle of some fungoid or yeast organism.

Swellengrebel [6] has reviewed the entire controversy and, following an investigation, reaches these conclusions:

1. In two cases *Blastocystis* was found where the presence of *Trichomonas* or *Chilomastix* could be excluded with absolute certainty. Consequently *Blastocystis* cannot be a normal developmental form of either.
2. In fresh stools *Blastocystis* is but seldom found to be alive and even

[6] *Parasit.* (1917), 9, 451.

when encountered in this state it dies quickly. After death the central sphere soon disappears.

3. The size of *Blastocystis* varies greatly and the larger they grow the smaller becomes the peripheral fringe of cytoplasm. Living blastocysts are relatively small and rich in cytoplasm.

4. The blastocysts of the cases mentioned here, although having some general characters in common differed much as to details of structure. This difference was especially marked when the associated parasites were different. No blastocysts were found without an associated parasite.

5. The occurrence of blastocysts in the stools of a man fed on milk and eggs only, and the presence of living blastocysts in the man's stools, exclude the idea of their being remains of solid food.

6. It is probable from the observation recorded in this paper, that "Blastocystis" is not the name of a zoölogical genus but of a peculiar form of degeneration to which representatives of different genera of intestinal protozoa may be liable. The resemblance seen in blastocysts from different sources which may lead to their being regarded as belonging to one species is easily explained by a convergence resulting from the parasites which produce blastocysts losing their characteristics during the process of degeneration.

7. No certain stages of sporulation were seen as described by Alexeieff, and the nuclear structure, although variable, never resembled that given in his description. It is therefore probable that Alexeieff's *Blastocystis enterocola* is different from the form described in man under the same name.

Chatton(9) also has done some recent work on *Blastocystis*, and while his preliminary paper does not directly bear on *Trichomonas*, his account, coupled with his figures, are very suggestive in connection with the work of Hadley, Kofoid and Swezy, and others. His work is largely confirmatory of that of Prowazek(43) on *Bodo* (*Heteromita*) *lacertæ*, but the host studied by Chatton was the Barbary gecko, *Tarentola mauritanica*. The appearances he notes are similar to those mentioned by other writers. He failed to secure a glycogen reaction in the vacuole whose contents he assumes to be of protein nature. Alexeieff laid some stress on the presence of glycogen in determining the bodies to be of blastomycetic nature, but it must be borne in mind that the cysts of *Entamœba* at times contain glycogen. Chatton describes the formation of flagellated cells within the microspheres. He has also seen the coupling of these flagellates, a performance probably similar to that described by Hadley, but he did not see subsequent fusion. Therefore he thinks the existence of copulation at this stage very probable.

Turning to trichomonad infections in man, there seem, in view of the foregoing, to be at least two important observations: namely, those of Castellani on *Entamœba undulans*(7) and of Gauducheau on the unity of *Trichomonas* and *Entamœba*.(16)

This again gives rise to the question as to whether *Trichomonas* as it appears in the intestinal tissues of man, if it does, so closely resembles *Entamœba* as to have been mistaken for the latter by the skilled observers who for years have been studying sections of the human intestine taken from cases of dysentery. Hadley's sections of the turkey cæcum ought to throw considerable light on this matter.

Castellani described his organism in 1905 as *Löschia undulans*. Gauducheau has reported a similar organism in the fæces of dogs. Wenyon(50) has already called attention to the similarity that *Trichomonas* bears to *Entamœba undulans* when the former is casting off its motile apparatus and assumes the amœboid form. The case reported by Castellani was in a European planter of Ceylon, who gave a history of entamœbiasis of the intestine and liver. His stool contained, in addition to the organism described by Castellani, *Entamœba* and *Trichomonas*. The organism measured 12 to 30 μ, but smaller forms were occasionally encountered. They had no flagella. There was a distinct undulating membrane along one border. Long, straight, finger-formed pseudopodia were rapidly extended and retracted, one at a time. No ecto- or endoplasmic differentiation was noted. The cytoplasm was finely granular and contained bacteria and a noncontractile vacuole.

There now seems little doubt that Castellani's *Entamœba undulans* is *Trichomonas*. The appearances described by him coincide so closely with developmental changes that have been seen in *Trichomonas* as practically to banish doubt on this point.

In the general consideration of these matters it seems worth while in passing to mention briefly Ijima's *Amœba miurai*, described by him(23) and by Miura.(37) It is not by any means certain that the bodies described by these two Japanese workers were living organisms. Many writers voice the opinion that they were tissue cells present in a serous exudate. In any event, the bodies were spherical or ellipsoidal. One end bore a small protruberance from which sprang several filamentous processes described as pseudopodia, but which more closely resembled cilia. The whole body measured from 15 to 38 μ. The cytoplasm was granular with no ecto- or endoplasmic differentiation. It contained several vacuoles, none of which was contractile. One to three nuclei were demonstrated on the addition of acetic acid. The bodies were discovered in the serous fluid of a young woman, who had died of pleuritis and peritonitis endotheliomatosa. Similar forms appeared two days before death in the bloody stool of the patient. Amœboid motion was not noted.

Gauducheau,(16) in 1912, announced his belief in the identity of *Entamœba* and *Trichomonas*. His observations apparently were made on an organism similar to that described in 1907 by Billet under the name *Trichomonas dysenteriæ*. Gauducheau claims to have isolated and cultivated *Entamœbæ* from a case of dysentery in man. He describes the organism as reproducing by multiple budding and giving rise to large, branched plasmodia. Cultivation he found difficult on nutritive agar sown with cultures of the *Bacillus coli* group. The organisms gave rise to spirocha te-like bodies in the culture. The ectoplasm was clear and formed pseudopodia resembling an undulating membrane, and endogenous buds were formed within the amœba. In the intestines of man and dogs, which had been injected with cultures of these amœbæ, Gauducheau reported finding all the intermediate stages between *Entamœba* and the flagellate. In the human bowel he found branched plasmodia from which flagella protruded in a manner similar to the appearance in his cultures. The flagellate, he states, develops to an amœba, and therefore he concludes the identity of *Entamœba* and *Trichomonas*. This caused him to fix three stages in the cycle of the parasite: 1, a cycle in the tissues of the host, the pathogenic phase; 2, a stage of saprophytism in the lumen of the bowel or in cultures during which time it lives on bacteria; 3, a stage when the completely developed organism lives free. He links Castellani's *Entamœba undulans* to this organism.

Mention has been made of the significance of diarrhœa in flagellate infection. Hadley and many others have noted the increase in numbers of the parasites under diarrhœal conditions. The doubt has lain as to whether the flagellates are the cause of the diarrhœa or whether the diarrhœa brings about an increase in their numbers. If the latter supposition is true, we have two possibilities: namely, either the change in composition of the fæces, through affording a more favorable environment in general, or through providing an increased food supply, accelerates division; or else the action of the diarrhœa is merely mechanical, tending to flush the flagellates out of the folds of the intestine. Barlow(2) found that twenty-five of his one hundred cases showed the presence of trichomonads in the stools after the administration of a purge. Of these cases, twenty-two had been reported negative on the routine examination of their stools. Alternate diarrhœa and constipation were present in fourteen cases, and in six of the latter there were other conditions adequate to explain their symptoms. Five cases showed

the presence of *Entamœba histolytica,* and three others were positive for an *Entamœba* of an undetermined species.

Barlow found that indiscretions of diet or the administration of cathartics would invariably cause the appearance of the parasites, and he concluded that the increase in numbers was the result and not a cause of the diarrhœa, and that the augmentation was dependent upon more favorable conditions for growth provided by the fluid stools. His study was based on *Trichomonas,* but it apparently was of the tetratrichomonad type, for he described his organism as having four flagella. As he was unable to demonstrate the axostyle in the examination of several hundred specimens, it is barely possible he was dealing with *Macrostoma* or some other species.

Woodcock(52) believes that *Trichomonas, Lamblia,* and *Balantidium,* like *Entamœba histolytica,* may occur without causing symptoms, "but on the other hand they are *potentially* harmful." He describes trichomonad stools as loose or thin diarrhœal, containing sometimes a little mucus and scattered pus cells. In a few of his cases manifestly dysenteric stools contained the parasites. The great majority of the stools were never of a dysenteric character, these being cases of pure *Trichomonas* infection. The diarrhœa, however, was troublesome and chronic and often resisted all treatment.

Castellani,(6) discussing the intestinal flagellates as a group, thinks that when present in small numbers they are probably harmless, but in large numbers they may give rise to symptoms. *Lamblia* he regards as the most pathogenic of all. The patient, he says, complains of the diarrhœa, and the yellowish stools may contain a little mucus. He has never found blood nor pus.

Wenyon(50) is another worker who states his belief that diarrhœa favors the multiplication of intestinal flagellates. Personally I have often seen them in formed as well as in diarrhœal stools, sometimes associated with *Blastocystis* or entamœbic infections, but more often not. I have seen only two pure infections with *Blastocystis.*

All this raises the question as to how the reactions of the environment affect the mode of life of the parasite in regard to nutrition and reproduction. Protozoölogists are familiar with the remarkable powers of adaptation shown by both the free-living and the parasitic Protozoa. The changes in mode of life and reproduction that follow the transfer of the malarial parasite from the warm environment of the human blood stream to the cold and otherwise different environment of the gut of

Anopheles are familiar. Similar differences are seen in the case of *Trypanosoma lewisi* as it lives in the rat and as it lives in *Ceratophyllus*. There are countless other examples, such as that afforded by *Euglena* on its removal from the influence of sunlight to darkness. Is it, therefore, wholly improbable that changes may occur in the intestinal tract that may lead to the substitution of a cytozoic or histozoic life for a cœlozoic life in the case of the intestinal parasites? *Balantidium* and even *Entamœba*, it is well known, may live in the intestinal tract for long periods of time without giving rise to symptoms, yet sooner or later they may invade the tissues. What are the conditions that lead to these diverse modes of life in the same organism living in the same site? May we not rather seek the answer in the host than in the parasite directly, and is it not something more than a mere lowering of vitality? Is there any good reason why changed conditions may not substitute tissue parasitism for lumen commensalism in the case of the flagellates? It seems improbable that the reactions of immunity as recognized in connection with bacterial and allied infections are to be considered here, for immunity as we know it in the Protozoa seems to be of an exceedingly low order.[1] Does it not seem that we are dealing with chemical affinities of a different nature, chemical reactions governing the regulation of the diseases of a characteristically nonfebrile character unaccompanied by phenomena of immunity? These phenomena need not be necessarily restricted to the metabolic chemistry of the parasite. They might be

[1] In connection with immunity problems with the Protozoa, it must always be borne in mind that in many instances animals recover from certain protozoal infections, such as coccidiosis, not through the development of any immunity, but solely through the normal life cycle changes of the parasite which develop the exogenous phases of the cycle. Once this border line is passed, the organism ceases to be infective to the host except through the original portal of entry. Gradually the schizogonous forms develop the propagative phase, until finally asexual reproduction—the only phase in which auto-infection is known to occur in such forms—has completely given way to sporogony. This, of course, leaves unanswered the question as to how we may account for persistent carriers of coccidial infection. This naturally presupposes either the continuance of the schizogonous cycle as the source of supply of gametocytes, or the reversion of the gametes or gametocytes back to asexual forms. The latter performance is so wholly at variance with established biological principles as to be, to say the least, rather improbable. On the other hand, we are led to wonder what are the conditions that bring about the prolonged series of asexual reproductions while the organism continues to produce oöcysts.

influenced by chemical changes originating in the cells and body fluids of the hosts.

The feeding habits of free-living forms may give a clue to the working out of this problem. They have been extensively studied by many workers, who have brought out important points. Whether the reactions in these phenomena are purely of a chemotactic nature or are combined with changes in surface tension remains to be proved. The significant thing, however, in connection with the theory I have just advanced is that these reactions in the case of parasitic forms *are not restricted to the protozoa, but may be participated in by cells belonging to the host.*

The most striking example of this that I can recall at the present time is that furnished by *Lankesterella* sp. This organism is parasitic in the nucleated erythrocytes of the frog. Neresheimer [8] has described and figured the penetration of the parasite into the erythrocyte. When the parasite and erythrocyte are separated by a distance about equal to the length of the parasite, amœboid movements on the part of the erythrocyte become evident, and eventually the blood cell throws out two long, pseudopodialike processes and, as Minchin remarks with characteristic felicity, "opens its arms, as it were, to the parasite, and engulfs it in a manner very similar to the ingestion of food by circumvallation on the part of an amœba." The parasite is then drawn into the body of the erythrocyte, which rounds out and resumes its normal form with the parasite in its interior. In this instance it must seem that the parasite gives off some substance that, even at a distance, awakens a reaction on the part of the host cell, which brings about its own destruction by aiding the entrance of the parasite. It is conceivable to me that under certain conditions epithelial cells may do the same thing.

The attraction of the gametes of protozoa is a process that is possibly in line with the above and is sufficiently distinct from anything shown by the bacteria to make us believe that in dealing with the factors that determine the relations between protozoan parasites and tissue cells we have something radically different from the conditions that govern bacterial infections.

Trichomonas flourishes only in an alkaline medium in the intestine. In the vagina it is found only in mucus having an acid reaction and is quickly got rid of by douching with alkaline

[8] *Arch. f. Protistenk.* (1909), 16, 187.

fluids.[9] If *Trichomonas intestinalis* is identical with *Trichomonas vaginalis* as some writers, notably Lynch,(27) hold, there is one example of adaptation to a changed environment. Lynch, in what is probably the first successful attempt to cultivate *Trichomonas* or experimentally to transmit it to a lower animal, cultivated *Trichomonas* from the vagina and mouth in acidified bouillon and injected a rabbit rectally, with the result that the fæces of the rabbit became soft and contained many trichomonads.

Ohira and Noguchi(40) on the other hand, cultivated the mouth trichomonad (a tetratrichomonad in their cases) in a slightly alkaline mixture of ascitic fluid and Ringer's solution, which they found much more favorable than acid media. They grew the trichomonads both at room temperature and at 37° C., which was 7 degrees higher than that at which Lynch was able to cultivate the organisms in his medium. Lynch observed no developmental phenomena in his cultures, but Ohira and Noguchi describe multiple fission and the budding off of the daughter cells one by one, as has been described by Kofoid and Swezy(26) in the case of *Trichomonas augusta.* Neither Lynch nor Ohira and Noguchi saw cysts. In speaking of *Trichomonas intestinalis,* Rhamy and Metts (44) say that the organism lives best in a neutral or slightly alkaline medium "and for this reason any tissue of low vitality will harbor them."

Disregarding for the moment the question as to whether the composition of the intestinal contents at any time influences the division rate of the organism, let us consider the possible factors bearing on the actual entrance of the flagellated organism into the epithelium itself. Several conditions may govern this: 1, the organism may mechanically bore its way in; 2, it may gain entrance through a break in the epithelium brought about mechanically by physicochemical changes or through the action of some other microöganism; or 3, it may secrete some cytolytic agent that attacks the epithelium. The fundamental question is, What stimulus is there that causes the organism to behave in this manner? That is the problem that presented itself to Hadley in his study and that he frankly states cannot be answered with any finality at the present time. In the case of the coccidia and other obligatory cytozoic parasites, it would seem that the organism characteristically and necessarily seeks a home within

[9] Escomel (*Bull. Soc. path. exot.* (1917), 10, 553) states that the reactions of *Trichomonas vaginalis* toward therapeutic agents are the same as those of *Trichomonas intestinalis.* He found that a 1 per 1,000 solution of metallic iodine (freshly prepared) would kill both species instantly. He bases his conclusions on studies in vitro.

the epithelium. But with the flagellates, whose habitat would certainly not seem to be normally in the tissues, the case is different. *Entamœba, Balantidium,* and the flagellates evidently find suitable conditions for life in both the lumen of the intestine and in the tissues and prosper to a greater or lesser extent in either place. If they show a preference, it seems to be in favor of the lumen of the intestine. With the coccidia the performance of tissue invasion is obviously in line with the regular life cycle; with the flagellates and possibly with *Entamœba* and *Balantidium,* tissue parasitism appears to be in the nature of a departure from the normal.

In *Coccidium,* for example, sporogony seems to fill the dual purpose of protoplasmic rejuvenescence (through fertilization) and the dissemination of spores. It seems to supervene upon sexual maturity and lowered protoplasmic vitality, and yet cases have been reported of chronic coccidiosis, in which the host became virtually a carrier, producing a constant stream of spores of the parasite, which obviously meanwhile continues its schizogonous cycle. Is this a matter of chemical adjustment between host and parasite?

Calkins's classical experiments with *Paramœcium* show the effects of artificial stimulation on the metabolism and reproductive rate of Protozoa and are merely an illustration of the effects that may be produced upon a protozoön by altered environmental conditions. These chemical changes may be, in the future, found to exert a more profound influence on the activities of parasites than we now realize. In 1914 I suggested(21) the possibility that relapses of malarial fevers might be brought about by a transient glycæmia, the blood sugar furnishing the rejuvenating stimulation. At the time I had in mind the work of Calkins on *Paramœcium* and that of Bass and Johns on the cultivation of *Plasmodium.* This view has been more recently expressed by Calkins,(5) who has suggested that the change may be, as I myself have long held, in the nature of the equivalent of fertilization, a process of the restoration of lost vitality, a substitution of a labile for a stabile condition of the protoplasm. Experimental evidence, as I have stated, points strongly to the belief that such a condition of protoplasmic rejuvenation can be brought about artificially within certain limits.

While we have so far no direct evidence of the invasion of the human tissues by the intestinal flagellates or flagellates of that type, unless we include Ross's observation of "cercomonads" having several flagella and an undulating membrane and tricho-

monads in some cutaneous ulcers, we have considerable evidence that it may occur under certain conditions in the lower animals.

Intestinal parasites representing genera frequently seen in man do, in the lower animals, pass from the lumen of the intestine into the blood and lymph on the development of certain pathological conditions in the host. This fact was early noted by Danilewski in the case of *Hexamitus*, a flagellated parasite in the intestinal tracts of tortoises and frogs. The animals in question had long been in captivity. They showed signs of distress and exhibited œdematous swellings in the muscles and transudation of lymph into the peritoneal cavity. Microscopic examination showed the presence of the protozoa in the blood, lymph, œdemata, and transudations. Plimmer(41) has reported a number of similar cases occurring in reptiles and batrachians in the London Zoölogical Gardens. He found both *Hexamitus* and *Trichomonas* in the blood. Plimmer holds that the presence of intestinal parasites in the blood stream is to be associated with definite and recognizable lesions of the intestinal wall. It is fairly well established in connection with the parasitic protozoa that in some cases a parasite, which may be perfectly or relatively harmless when the host is free, may become pathogenic or even lethal when the host is in captivity.

Wenyon(50) points out that intestinal flagellates, like the intestinal bacteria, occasionally invade the tissues shortly before or after death. He attributes this to diminished resistance on the part of the intestine, which permits the passage of organisms that normally live in the lumen of the gut. Diminished resistance is a broad term that covers up considerable uncertainty. Is it wholly, in this case, a matter of lowered resistance, are essential chemical changes involved, or do the processes of autodigestion of tissues of the alimentary tract, which may set in soon after death, play their part?

Wenyon quotes Basile, Gonder, and Stevenson on the matter of tissue invasion by the flagellates. Gonder recovered *Lamblia* from the blood stream of a fowl; Basile, *Lamblia* from the liver of a rat. The liver was dotted with white cysts containing the organisms. Basile inoculated a rat peritoneally with the contents of some of these cysts and later found *Lamblia* in the liver and mesenteric glands. Stevenson showed Wenyon sections of the cæcum of a mouse having definite lesions of the mucous surface that had been invaded by numerous trichomonads. *Lamblia* has been shown as invading the glands of the small intestine of the rabbit. Wenyon says:

> These are exceptional cases, but so long as they occur there is the pos-

sibility that the invading flagellates will give rise to symptoms of one kind or another. * * * If such invasion can occur [speaking of *Trichomonas*], probably through a surface broken by some other infection, or irritant such as sand, it is possible that the flagellates might aggravate the lesion or produce definite symptoms.

So much for *Lamblia* infections in the lower animals; but Fantham and Porter(12) carry the matter farther in their inquiry into human lambliasis. They say:

We may say at once that in both human and animal lambliasis stools, as well as at post-mortem examinations, erosion and distortion of the intestinal epithelial cells occurred, *owing to the direct suctorial action of the flagellate Lamblia.*[10]

Woodcock(52) holds in the case of flagellate infections in man that if the normal bowel condition becomes disturbed and its resistance lowered the balance between the host and the parasite is no longer maintained, and the parasites will, therefore, multiply more rapidly and in turn bring about a more intense reaction on the part of the host, establishing a vicious circle. He believes that the presence of a vast number of parasites in the intestine "with the concomitant production of waste metabolic substances, is sufficient to irritate the mucosa and give rise to deleterious and toxic effects." He adds:

Fortunately, the flagellates appear to be unable to penetrate the mucosa in the way that *E. histolytica* and *Balantidium* can (given favourable conditions), and therefore, never (?) produce true dysenteric symptoms, the derangement being generally limited to severe or mild diarrhoea.

Minchin(34) raises the old question as to whether the migration of the parasite, when the tissues are involved, is the cause of the diseased state or if the diseased state of the host gives the parasite a chance to spread to other parts of the body. Aside from that point, he makes the exceedingly pertinent remark that the fact that intestinal parasites can pass from the gut to the blood is of phylogenetic as well as of practical importance.

Walker(49) discusses the case of *Balantidium coli* infections in man. He speaks of the absence of liver involvement by this parasite, quoting the old case of Stockvis,(48) in which *"Paramaecium"* (*Balantidium* ?) was recovered from the sputum, the parasites presumably having come from the liver by way of the lung.

In connection with the involvement of the liver in entamœbiasis and balantidiosis it is customary to regard the route of invasion as lying through the portal circulation, the parasites entering the radicles of the portal vein. It must be borne in

[10] The italics are mine.—F. G. H.

mind, however, in connection with the powers shown by *Entamœba* and *Balantidium* to penetrate deep into the tissues of the intestine that the close apposition of portions of the colon to the surface of the liver make it possible that the liver may become involved as a result of the direct migration of the parasites through the tissues, a contingency that has been in the minds of certain pathologists for some time. The possibility of the conveyance of the parasites through the lymph channels must not be entirely overlooked. The likelihood of retrograde infection through the bile ducts is rather remote, although it is conceivable. Stress has been laid by some of the workers on the presence, more or less frequently, of *Balantidium* in the blood vessels and the lymph spaces, and Bowman(3) and Walker(49) both report the presence of *Balantidium* in the lymph glands of the mesentery.

Walker states his firm conviction that *Balantidium* will pass through the healthy epithelium. This process, he says, is not accompanied by necrosis or ulceration of the epithelium; it consists of the pushing aside or at the most a rupture of the epithelium. In every case, he says, entrance is through the epithelium and in no case within the tubules. Once within the tissues, the presence of the parasites is certainly associated with necrosis. Walker adds that the lesions and cellular reactions produced in the early stage before being complicated by secondary bacterial invasion are characteristic and are distinguishable from those due to bacterial infection.

In experimental work performed on monkeys, Walker found the epithelium intact "except for mechanical injury due to entrance of the balantidia or to minute hæmorrhages, but no exudate or ulcerations." He also concludes that lesions in the intestinal epithelium from bacterial infection or other causes are not only not necessary for the entrance of the *Balantidium* into the tissues, but that in none of the monkeys in which such lesions existed have the balantidia taken advantage of them to enter the tissues.

Manlove(28) cites two cases of balantidial dysentery in man, in support of Walker's contention that *Balantidium* may produce "abscesses" in the intestine that are sterile except for the presence of the protozoön. Walker mentions the fact that the *Balantidium* found in the pig, which he holds to be identical with that infesting man, seldom if ever penetrates the tissue of the pig intestine. On this point I can speak from experience, for I have found *Balantidium* in sections of the pig intestine

not only in the tissue, but in the blood vessels as well; in fact, the microscopic picture was similar to that seen in the case of human balantidiosis.

Walker also points out the fact I have mentioned that an individual may harbor *Balantidium* over long periods of time, apparently without sustaining harm. But sooner or later, he adds, the patient so parasitized develops balantidial dysentery. The periodic appearance of the parasites in the stools that he notes is a phenomenon seen frequently in connection with the intestinal Protozoa as a group.

The oral apparatus of *Balantidium* is similar to that of a number of free-living Heterotrichida, and indeed *Balantidium* is suspected of being capable of existence free in pond water. The oral apparatus of this organism does not seem, at first glance, particularly to adapt it for penetration of the tissues; and if we are to adopt the views of Walker and of Manlove, it remains to discover how this may be brought about.

Bowman quotes Glaessner(19) as having recovered a very active diastase and a fairly strong hæmolytic substance in extracts of *Balantidium coli* from the fæces. These ferments exerted no effect on dilute albumen, on peptone solution, or on fibrin. One naturally asks why they should and why they should be concerned in cytolysis. Any such supposition seems to me to be entirely untenable. It seems to be much more probable that Glaessner, after all, merely isolated some of the normal digestive ferments of the parasite. Both diastatic and hæmolytic ferments seem to be exceedingly necessary to the carrying out of the normal anabolic processes in an animal living in such an environment as that in which *Balantidium* lives. A diastatic ferment ("amœbodiastase") has been isolated from amœbæ. Proteolytic ferments have been isolated from other protozoa, and their action has been the subject of considerable study by Nirenstein, Greenwood, Metchnikoff, and others.

It is still an open question whether *Entamœba* and *Balantidium* enter the tissues *through* or *between* the cells and whether the entrance is effected *mechanically* or by the aid of *cytolysins*. Manlove(28) quotes Crowell [11] as stating his belief that the question of the essential pathogenicity of *Balantidium* is not yet absolutely settled. As to the invasive power of the organisms, Manlove says:

> Doctor Crowell expressed the theory that it seems reasonable to conclude that some substance (toxin?) originating from the balantidia is able to

[11] Crowell, B. C., personal communication.

produce necrosis of the tissues, and the inflammatory phenomena that occur later are the result of the action of the accompanying bacteria. Also one could imagine that this hypothetical toxic substance from the balantidia produces a cytolysis of the epithelial cells of the mucosa with which they come in contact and so forms a portal of entrance to the deeper tissues for the organisms.

Restating this view to me, Crowell [12] holds that it is logical to suppose that, if the balantidia are capable of producing within the tissues a substance that brings about necrosis, this same substance might be employed in the initial penetration of the intact epithelium.

However, it may be pointed out that a fundamental objection that might be opposed to this hypothesis is the fact that substances that are capable of producing marked injury to the unprotected connective and other tissue elements of the submucosa may have no effect whatever on the healthy epithelium, the chief function of which is constantly to protect the underlying tissues from substances that would be injurious. To injure by a toxic substance the intact epithelium would, it seems to me, require a stronger toxin than the changes produced, once having effected penetration, would indicate. That is, of course, provided there is no specific epitheliolysin, of which there is no evidence. On the other hand, much of the evidence I have already quoted points to the *mechanical* invasion of the intestine, rather than to the employment of cytolysins. Walker has stated his belief that entrance is accomplished by the pushing aside or rupture of the epithelium, and similar evidence in the case of *Trypanosoma lewisi* seems to have been produced by Minchin and Thomson (36) and by Nöller, (39) in the case of *Trichomonas* by Hadley, and in the case of *Trypanosoma gambiense* by Hindle. (22)

Carrying the case to the parasitic Rhizopoda, the active, powerful pseudopodia, formed mainly of ectoplasm as seen in *Entamœba histolytica,* are in marked contrast to the relatively blunt, inactive pseudopodia of *Entamœba coli,* which contain comparatively little ectoplasm, and suggests the possibility that it is this difference in the structure of the pseudopodia that enables *Entamœba histolytica* to push its way between the cells. Are cytolytic agents necessary here? Or, in the case of *Balantidium,* are the cell membranes or bridges between the cells able to withstand the powerful currents set up by the cilia

[12] Crowell, B. C., personal communication.

and membranelles of the adoral zone of the organism when closely and persistently applied to the surface of the tissue? Once the entrance is forced, it would be about as easy for one of these ciliates to worm its way in as it would be for an amœba. Their bodies are plastic to a degree that at times superficially resembles pseudopodia formation, and they accommodate themselves to every obstacle they meet as may be readily seen by any one who will take the time to study a cover slip preparation from a case of *Balantidium* infection containing actively moving parasites.

After having gained entrance to the tissues, it seems to me for the present reasonable to believe that the nutrition of these organisms continues to be holozoic within the tissues, so we are left to speculate as to whether necrosis in the tissues, where involvement is unaccompanied by the presence of bacteria, is due to the secretion by the organism of some special cytolytic ferment, or whether it is brought about by the action on the tissues of the normal katabolic products of the parasites, a theory which seems to me not wholly devoid of reason. This may also apply as well to *Entamœba histolytica* as to the flagellates and ciliates.

But aside from tissue penetration, the situation contains further inconsistencies. *Balantidium*, from such reports and personal experiences as are available to me at this time, seems to be more frequently seen in the blood vessels of the intestine than *Entamœba*. In spite of this, however, *Balantidium* is never found in the liver; whereas *Entamœba* is very frequently found there and in other parts of the body. Why is this?

May we not find our answer in the general tendency of the flagellated and ciliated protozoa, spermatozoa, and the like to swim *against* the current, whereas the relatively passive entamœbæ are carried willy-nilly by any current to a point where they meet with some obstruction, finally to lodge and reproduce, provided the conditions are favorable? *Balantidium* and *Trichomonas*, with their powerful, well-developed motile organs, would surely have less trouble in stemming the tide of the slow-moving venous and lymph circulation than even the poorly equipped spermatozoön has in breasting the current of the ciliated Fallopian tubes. And yet the population of the world attests the success of the spermatozoön, and the absence or comparative absence of extra-intestinal infections with *Balantidium*, *Trichomonas*, *Lamblia*, and the like seems to betoken equal

success on the part of these organisms in avoiding being carried away. This apparently constitutes an application of the principle of the "avoiding reaction" as described by Jennings(24) in his studies on the tropisms in the Protozoa. In the smaller vessels of the intestine, aside from the obstruction afforded by the walls of vessels having a caliber smaller than the diameter of the parasite, the flow is probably too slow to bring about the degree of stimulation necessary to excite this reaction. The parasites would probably be prevented by the excessive blood pressure from even entering the arteries, but granting that they might do this, the stimulation would probably pass above the *optimum*, and the organisms would be passively swept back.

But in the larger radicles, where the current is *away* from the intestines, it is conceivable that the flow would be of about the right intensity to cause the parasites to about face and turn "upstream." At the same time it would be strong enough to sweep the entamœbæ toward the liver. If this hypothesis will hold, it would be added evidence to the effect that in hepatic involvement the main path would be through the portal circulation. Lastly, however, it must not be forgotten that there still remains the possibility that the balantidia may reach the liver, but find the conditions for continuation of life unsuitable at that site. If Stockvis's observation is correct, that contingency seems to be ruled out. The possibility of the conveyance of the cysts of *Balantidium* to the liver through the blood stream appears to be too remote for consideration at this time. There is no definite proof that *Balantidium* forms cysts in the tissue, and it would require to be shown that the cysts would open under the influence of any agent that is likely to be present in the liver. Normally these cysts may be expected to open under the influence of some digestive ferment only, though it is well known to protozoölogists that certain protozoan cysts will open under the influence of ferments secreted by bacteria or through external or internal influences of an uncertain nature. The cysts of the intestinal parasites, however, appear to be adapted to the action of the intestinal juices.

Turning back to the flagellates, let us review what evidence we actually have of their tissue-invading powers.

Minchin and Thomson, in their great memoir on *Trypanosoma lewisi* and its relations to the rat flea *Ceratophyllus fasciatus*,(36) describe in considerable detail an intracellular stage in the stomach of *Ceratophyllus* following the penetration of the epithelium. They were not so fortunate as to observe the actual

penetration of the cell by the parasite, but Nöller, (39) who did, states that he saw—

> a trypanosome of which the pointed hinder end had already penetrated into an epithelial cell. The flagellum-bearing anterior end beat violently and incessantly, whereby the trypanosome penetrated further and further into the cell. After I had watched this spectacle for about five minutes the trypanosome, which had so far penetrated into the cell as far as the middle of its body, suddenly shot into the cell and stirred up the granular cell-contents by its lively movements. Since, however, the cell was torn on its opposite side the trypanosome shot out of the cell again.

In discussing the type of cell attacked by the trypanosomes, Minchin and Thomson are somewhat in agreement with the observations of Walker. They never saw intracellular stages of the trypanosomes in the cells of the epithelial crypts. The trypanosomes never occurred in any cell that was not definitely a part of the general epithelium, and the appearances they saw seemed to suggest that "the attack is usually made on the side of the cell; the occasional, though rare occurrence, however, of intracellular stages in quite young cells, shows that the trypanosomes can penetrate into epithelial cells before the separation between them has developed."

Of course, the general character of the epithelium of the alimentary tract of the flea probably differs in important details from that of the epithelium in the human digestive tract, but the conditions that exist in certain cells and that may profoundly influence tissue invasion are suggested in the following paragraph from the paper of Minchin and Thomson:

> The trypanosomes attack by preference the fully-formed, but still young and vigorous cells, which may contain granules of the normal type and even yellow bodies, but no fatty deposits; cells which may be well characterized as adolescent in type, and which, stain a clear, light-grey with iron-hæmatoxylin after Flemming-fixation. It is in such cells that the earlier stages of the intracellular multiplication are to be found in flourishing condition and often in considerable numbers; but if the trypanosomes are numerous the cell soon becomes exhausted.

Evidence of the possibility of the transmission in man of *Trypanosoma gambiense* by coitus was brought forward by Koch, (25) in 1907, and the transmission of *Trypanosoma equiperdum* in dourine has long been an established fact, although trypanosomes have not been demonstrated in the seminal fluid, and the possibility of even slight abrasions of the mucous membranes of the contracting parties still remains.

Hindle (22) has made a study to determine the possibility of the entrance of trypanosomes through the intact skin and mucous

membranes. Using *Trypanosoma gambiense,* he was able to secure infections per os, care being taken to prevent the possibility of producing lesions in the mouths of the rats used. Attempts to secure infection by coitus between rats were unsuccessful, but careful experiments in infection through the vagina were successful. Infection was also secured by applying the infected blood to the unshaved skin of rats, and Hindle concludes that *Trypanosoma gambiense* is able to penetrate the sound mucous membrane and the undamaged skin.

It seems from this that certain, at least, of the flagellates experience no difficulty whatever in penetrating cells and tissues. Minchin and Thomson have shown that apparently the side of the cell is the most vulnerable point. Furthermore they cite the adhesive properties of the flagellum under certain circumstances when the organism may attach itself to the epithelial surface by its flagellum and then bore its way in, posterior end first. Might not a similar action be possible in the case of *Trichomonas,* where the axostyle would play an important part? This, of course, apart from the condition where *Trichomonas might* assume an amœboid form, as described by Castellani(7) for *Entamœba undulans,* and bore its way in by its long, powerful pseudopodium. *Crithidia* is known to attach itself to epithelium by its shortened flagellum. This would give us, then, three separate relations existing between intestinal flagellates and the epithelium:

1. Close application to the surface of the epithelium without actual penetration. *Lamblia.*

2. Attachment by a flagellum, which may penetrate the cell membrane. *Crithidia.*

3. Actual penetration of the cell and entrance of the entire organism into the cell body and subsequent liquefaction of the cell contents. *Trypanosoma, Trichomonas, Hexamitus,* and possibly *Lamblia.*

The amœboid, flagellated, and ciliated protozoa of the alimentary tract represent a group standing apart from the other protozoan parasites. For the most part they are, in regard to structure, mode of life, and nutrition, not very different from free-living forms we may find in almost any mud puddle or watering trough. That they show certain tendencies toward development to obligatory tissue parasitism cannot be denied, but they can scarcely be said to have "arrived."

The amœboid forms as represented by the entamœbæ are strikingly similar in structure and mode of life to *Vahlkampfia*

and other small free-living amœbæ, while *Chlamydophrys stercorea* closely resembles some of the free-living testate forms.

The flagellates exhibit elaborate motile apparatus and, in many cases, well-developed mouth parts for the ingestion of food.

The ciliated parasites such as *Balantidium, Nyctotherus, Diplodinium, Ophryoscolex,* and the like not only resemble free-living forms in regard to their motile and food-getting organs, but many of them are highly organized in other ways, through the possession of neuromotor apparatus. *Opalina* seems to constitute an exception to the rule.

The absence in most parasitic forms of the contractile or excretory vacuole, which is cited by many as a characteristic of the parasitic protozoa, is not by any means necessarily an adaptation purely to a parasitic mode of life. It is an adaptation, to be sure, but parasitism is not the only thing that determines it. The marine species of protozoa for the most part do not possess contractile vacuoles. The gradual transfer of a marine species to a fresh-water environment will frequently cause it to develop a contractile vacuole, which it will lose on its return to salt water. So that the possession or absence of this organelle seems to be governed largely by the osmotic tension of the medium in which the organism finds itself.

In other words, Are these forms to be considered as on the same plane with the blood and obligatory tissue parasites as the trypanosomes and coccidia? It would seem not. The intestinal flagellated and ciliated protozoa of man, with the exception of *Lamblia,* do not show evidences either of a high degree of adaptation to a parasitic mode of life or evidences of structural degeneration as a result of parasitism. They have not found themselves. They are, in a measure, creatures of impulse subject to the play of natural forces that are yet to be understood, responding to frequently changing stimuli by varied reactions that are the despair of the physician and parasitologist alike. They have yet to settle down and behave with the almost mathematical regularity that we have grown to expect of their more conventional brethren, such as the coccidia and trypanosomes.

With the flagellates it appears that tissue parasitism is a departure from the normal and with only lesser force would this seem to apply to *Balantidium* and *Entamœba.* It is perfectly clear that many of these parasites may live in the intestinal tract over long periods of time without causing trouble—perhaps they may never cause trouble during the life of the host. On the other hand, the day may come when some con-

dition arises—lack of resistance on the part of the host or some other change in the host that intervenes to endow the parasite with new powers, as has been hinted by Hadley—and the parasite strikes boldly out into a new field, the tissues. What these conditions are remains to be determined. That they can be explained on the same ground as we explain bacterial invasions has been a popular supposition, which it seems to me would be wise for us to abandon for the present at least and to look for something new. We now know considerable about the reactions between *animals* and *plants* (bacteria), and it seems about time we added to our knowledge regarding the reactions between host *animals* and parasitic *animals*.

Other points of inquiry bear on the discovery of the conditions under which a parasite is harmless to its host when the latter lives free but becomes pathogenic or even lethal when the host is in captivity and of the possible free life of certain intestinal protozoa as suggested by Rhamy and Metts, Escomel and Smithies, and supported by laboratory experimentation.

The time has arrived when workers in the field of parasitology should fairly face the situation presented by the intestinal flagellates. They have been under suspicion for many years, during which time there has been gradually accumulating a mass of evidence against them. No progress will be made if we are to continue to employ, as our criteria of pathogenicity or non-pathogenicity, the presence or absence of blood and pus in the stools. Among the possible effects that may be produced by these organisms may be mentioned:

1. The production of antigrowth vitamines or growth-inhibiting substances, as suggested by Gibson.

2. The production of substances directly toxic.

3. Unfavorable effects upon the host through the liberation of the products of metabolism of the parasite.

4. Mechanical irritation of mucous surfaces by the parasites when present in large numbers.

5. Interference with absorption in the intestine through the adherence of large numbers of parasites to the surface of the epithelium, as in the case of *Lamblia*.

6. Actual invasion and destruction of the tissues with all its concomitants and sequelæ.

Analysis of the work of Castellani on *Entamœba undulans*, of Gauducheau on *Entamœba* and *Trichomonas*, and of Hadley and others on the tissue-invasive power of *Trichomonas* and the

other intestinal flagellates seems to be suggestive of what may be expected in man in regard to these parasites.

Next in order appears to be the desirability of attempting to explain the conflicting opinions expressed by different authors regarding the pathogenicity of the intestinal flagellates. It has been shown that some workers regard these forms as harmless or capable, at the most, of giving rise to nothing worse than diarrhœa, while others frankly express the belief that they may produce dysentery—actual lesions of the bowel. Is it possible that we have here different strains of the same organism, some showing and others not showing tissue-invasive powers—a condition somewhat resembling the relation between *Entamœba histolytica* and *Entamœba coli?*

Another problem is that of cross-infectivity. Many of the genera found in lower animals are found in man. Are the species found in the lower animals capable of life in man? The rule is known to apply in the case of *Balantidium*, it seems to be the case with *Lamblia*, and Lynch has produced evidence to show that the rat is a true host and not merely a carrier of *Entamœba histolytica.*

The actual invasion of the tissues of the human intestine by the flagellated parasites remains to be demonstrated, but it may have occurred and we may have passed it by. It seems conceivable, as I have suggested, that *Trichomonas* in the tissues may so closely resemble *Entamœba* as to have been mistaken for the latter. Evidence that tissue invasion by these same parasites occurs in the lower animals seems to be sufficiently convincing. However, it is still to be proved how this takes place. To my mind the evidence, so far at least as the flagellates and ciliates are concerned, seems to favor mechanical penetration of the tissues rather than entrance with the aid of cytolytic agents. But it is also a fact that necrotic changes in the tissues may be associated with the presence there of the parasites. These changes might be due either to definite cell-destroying ferments, or might simply be the result of the action of katabolic products of the parasites that happen to be toxic to the cell elements. From the evidence, cytolytic ferments appear to be unnecessary in many cases, certainly with many of the flagellates and possibly in the cases of the ciliates and amœboid forms.

But this should not preclude a study of the cytolytic enzymes that may be produced by these organisms. If they exist, the investigations should include an inquiry into the conditions under which they are formed with a view to discover whether they are constantly secreted or are produced only under special in-

fluences either existing at some definite period of the life cycle of the parasite or originating in the host.

Enough has been shown effectually to despoil *Trichomonas* and its cousins of the reputation for harmlessness to man they have previously had. It remains to determine some effective remedy for them.

With this should be coupled a closer study of the bionomics of the protozoa parasitic in the alimentary tract. It is only through such a study that we can hope to secure the knowledge of the intricate and often seemingly anomalous relations between these parasites and their hosts that will lead us to a better understanding of their activities and that will make it possible to discover the appropriate means for controlling the conditions to which they give rise, from the viewpoints of both cure and prevention.

ADDENDUM

Since the foregoing was written, several papers dealing with various phases of the problem of flagellate infection of the intestine have come to hand. To deal with them would involve the resetting of a considerable portion of this paper. Particularly worthy of the attention of those who are interested in the subject is the paper of Chalmers and Pekkola, [13] in which they discuss *Chilomastix mesnili* and give a systematic review of the Tetramitidæ. Gäbel in his paper on the pathogenic flagellates [14] gives an extensive bibliography of the literature on flagellate diarrhœa.

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ILLUSTRATION

FIG. 1. Growth curves for dogs fed on fresh and autoclaved milk.

PRELIMINARY REPORT ON VARIOUS METHODS OF SERUM APPLICATION IN BACILLARY DYSENTERY[1]

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TWO TEXT FIGURES

Since the announcement to the world of the specific therapy of bacillary dysentery introduced by Shiga in 1898, this form of treatment has fallen into a state of apparent lethargy in the hands of the medical profession probably because of the failure of other observers to secure confirmatory results. It is only during recent years that the "renaissance," so to speak, of the biological applications of this treatment has attracted many followers. Leading observers who have contributed much to the knowledge of this subject are Shiga, Kruse, Rosenthal, Kraus and Doerr, Rosculet, Vaillard and Dopter, Irimescu, Karlinski and Ludke.(2)

The specific treatment is based on purely biological principles. By immunizing animals, Shiga, the pioneer in this field, could produce sera with which he was able to decrease the mortality rate of endemic dysentery in Japan from 35 to 9 per cent.(4) During an epidemic in 1905 in Rumania Rosculet used prophylactic injection of serum in 18 persons, while 18 other persons exposed under the same conditions were used as controls. While none of the exposed persons who received serum showed any symptoms of the disease, the controls developed bacillary dysentery. Bahr has claimed that polyvalent sera are both antitoxic and bactericidal.(1) These facts themselves speak highly for the specific treatment of the disease and its bright future in connection with the treatment of the disease.

Other investigators, however, have pronounced the serum treatment of bacillary dysentery a failure. It is probable that (a) the serum at their disposal was defective, (b) the treatment was begun too late, or (c) the diagnosis had not been confirmed bacteriologically and the etiology of the disease was not established. Under such circumstances, what beneficial effects could

[1] Read before the Manila Medical Society, December 3, 1917. Received for publication January 17, 1918. The serum used in this work was supplied by the Bureau of Science.

be expected from the administration of antidysenteric serum? On the other hand, experiences of those observers who have made careful studies of serum therapy as applied to this disease speak in favor of this method of treatment.

According to the present communication the use of specific treatment seems to be an effective means of checking the progress of the disease in cases of true bacillary dysentery. Out of 20 cases that I have so far had the opportunity of treating, only one has died. Different methods of administering the serum, namely, intramuscularly, intravenously, and per rectum (serum enema), have been employed.

During September, October, and November, 1917, there were admitted to the medical wards of the Philippine General Hospital 20 cases of bacillary dysentery, which were treated with serum. Diagnosis in all these cases was based upon clinical symptoms and laboratory examinations. Out of 17 cases in which cultures were made from the fæces, six were negative. Of the 11 cases that were found to be positive, two were of the Shiga and one of the Flexner type. Further differentiation of the rest was not made owing to the fact that there were no media available for this purpose.

Of these 20 cases, five were treated medicinally combined with intramuscular injection of serum with one death; six cases intramuscularly with no deaths; three cases treated both with intramuscular injections and antidysenteric serum per rectum with no mortality; three cases treated solely with serum per rectum with no deaths; and finally three cases treated intravenously, with no deaths (see tables).

TABLE I.—*Drugs and serum intramuscularly.*[a]

Case.	Name.	Age.	Sex.	Result.	Culture.	Complication.	Remarks.
1	C. R.	26	F	Recovered	—		
2	R. C.	18	M	do	+		
3	E. G.	17	M	do	No media.		
4	J. G.	25	F	do	+		
5	J. D.	29	F	Died	—	Lobar pneumonia.	Patient had been sick sixteen days and was in collapsed condition on admission. No autopsy performed.

[a] In the culture column in this and following tables — means dysentery bacilli were not obtained in cultures, though sought for; + means dysentery bacilli were obtained in cultures. When the type of dysentery bacilli isolated was identified, it is so indicated (see text). In each case, search was made for amœbæ.

TABLE II.—*Serum intramuscularly alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.
6	G. S.	22	F	Recovered	+
7	E. R.	22	F	.....do	—
8	C. E.	42	M	.....do	+
9	F. B.	22	F	.....do	No media.
10	A. L.	21	F	.....do	Do.
11	L.	26	M	.....do	—

TABLE III.—*Serum intramuscularly and per rectum.*

Case.	Name.	Age.	Sex.	Result.	Culture.
12	E. C.	19	M	Recovered	+
13	E. A.	27	M	.....do	+ (Shiga)
14	J. M.	24	M	.....do	+

TABLE IV.—*Serum per rectum alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.
15	S. T.	45	M	Recovered	+
16	G. R.	14	F	.....do	+ (Flexner)
17	G. R.	16	M	.....do	

TABLE V.—*Serum intravenously alone.*

Case.	Name.	Age.	Sex.	Result.	Culture.	Remarks.
18	E. B.	20	F	Recovered	Shiga +	1 day before treatment.
19	A. M.	30	M	.....do	—	10 days before treatment.
20	Z. C.	37	M	.....do	—	Do.

Table I is intended to show the cases in which drug therapy was combined with serum treatment. With the exception of a single case that died, having been admitted to the hospital in a condition of collapse at the end of sixteen days of sickness, all the other cases were of moderate severity. The average duration of treatment, excluding one case that succumbed, was four and one-half days.

Table II shows the cases treated intramuscularly with serum alone. In this group one case (8) was severe, the rest were only moderately so. The average duration of treatment was four days.

Table III presents cases treated with serum intramuscularly and per rectum. These were all severe, a fact which very likely

is responsible for a longer average duration of the disease, namely, seven days. Case 12, for instance, was having almost continuous bowel movements consisting of a few drops of pure blood every few minutes. The patient was able to count only large evacuations; he gave thirty-five as the number of evacuations during twenty-four hours, which is probably five times less than the actual number of bowel movements.

Table IV presents cases treated with serum per rectum only. All these cases were moderately severe. The average duration of treatment was four days.

Table V presents cases treated intravenously with serum. These cases were all severe. Patients were able to count only large evacuations, for they were passing stools consisting wholly of blood almost continuously. The average duration of treatment was five days.

The serum per rectum was given in the following way: The patient is in the knee-chest position. The injection of the serum was preceded by a cleansing enema of 1.5 per cent solution of sodium bicarbonate; this was followed by another enema of starch solution with a few drops of tincture of opium (60 cubic centimeters with 10 drops of tincture of opium) to diminish the irritability to the intestine; a half hour later the serum was given per rectum. The amount of serum used was from 30 to 50 cubic centimeters daily, depending upon the severity of the case, although the serum can be frequently given without any danger and in larger doses.

The intramuscular administration of serum was done with the usual aseptic precautions. Twenty cubic centimeters of the serum were given twice a day, usually injected into the buttock. Larger doses may be given, depending, of course, on the severity of the case. Willmore advises the injection of 80 to 120 cubic centimeters daily in desperate cases;(7) Lukis administers 20 cubic centimeters four times a day every six hours.(3) Bahr emphasized the necessity of first cleansing the bowel by the use of a saline purgative, preferably sodium sulphate, so as to accelerate the repair of the ulcerated mucous membrane and also to eliminate unabsorbed toxins, which are responsible for the symptoms of collapse frequently met with in acute and severe cases.

Last of all is the use of serum by intravenous injection. It was done by the closed method and under rigid asepsis. Usually the median basilic vein was selected, being commonly prominent. To avoid anaphylactic symptoms, one cubic centimeter of the

serum may be previously injected intravenously about six hours before the full dose is given. My dosage was 10 cubic centimeters every other day, although Sandwith has given 20 cubic centimeters (of the Lister Institute preparation) daily to adults and 10 cubic centimeters to children.(6)

It is not the purpose of this paper to discuss the clinical symptoms of the disease, for these are generally well known. The main point that I wish to bring out is the broader application in the field of medicine of a therapy based on scientific principles.

Again I have to state that it has long been definitely known that specific bacteria are the causative factors of this disease and that they produce definite pathological changes, particularly in the large intestine. Observations of those writers who have made careful studies of this disease have led to the conclusion that the toxins act locally and, when absorbed in large amount, produce toxæmia. Experiments on animals support these views. Injection of dysenteric toxins into susceptible animals produces similar symptoms and anatomical changes in the large intestine to those observed in human beings. These are the findings of Flexner and Sweet and of Doerr.(2) Doerr, however, could save the animals from the effects of a lethal dose of toxin by previous injections of serum. Similar experiments were reported by Todd and later by Vaillard and Dopter.(2) Sandwith states that serum is both antitoxic and bactericidal.

In view of the established facts set forth by authentic observers on this subject, the local use of the serum seems, to my mind, not to be unscientific. Considering well the morbid changes in the large intestine, where acute inflammation and ulcerations are taking place, and recalling that it is the site where the virus flourishes the best, continuously elaborating the toxins, we can see at once the working basis of the local application. As it has been found that the serum is both antitoxic and bactericidal, it would mean, then, the neutralization, locally, of the unabsorbed toxins and the decreased vitality of the virus, if not its actual death.

As to how far these aims are accomplished, I am not yet in a position to state; but it suffices to say that twenty-four hours after the administration of serum per rectum the patient feels a marked alleviation of the local symptoms. The colicky pain is markedly diminished; the stools may still be very bloody, yet greatly decreased in number; and the temperature is lower. During successive days the stools gradually become less bloody

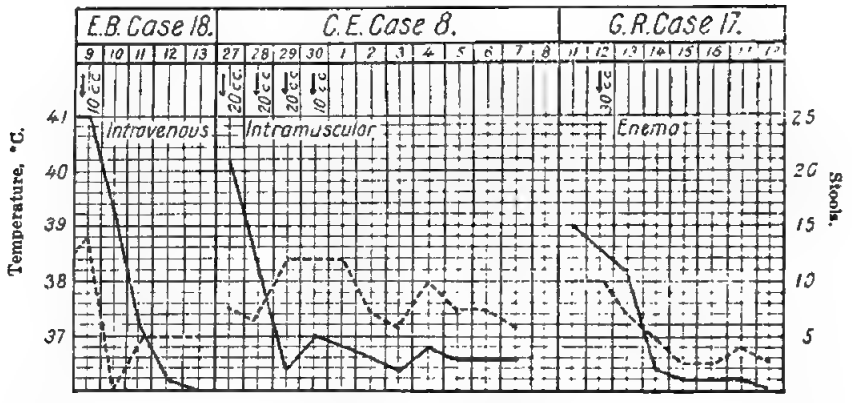

FIG. 1. Cases 8, 17, and 18. Solid line, stools; broken line, temperature. One square is equivalent to one stool.

and mucoid, then feculent, then of a soft consistence, finally becoming normal. I am fully aware of the drawbacks of this method. The serum may not reach the whole area of tissue involved, or it may fail to neutralize the toxins already absorbed in the system, as has been observed in cases 13 and 17. In these two cases charts were made, showing the comparative effects of the serum given per rectum both on the temperature and stools. It is an illustration of a case where serum per rectum is a failure, because the toxin has been already absorbed by the system.

The advisability of the administration of serum by intramuscular injection can be readily seen. Knowing that the toxins of the microörganisms have been already absorbed, naturally we expect the neutralization of the poisons by the serum, which contains specific antibodies. The results are gratifying. If

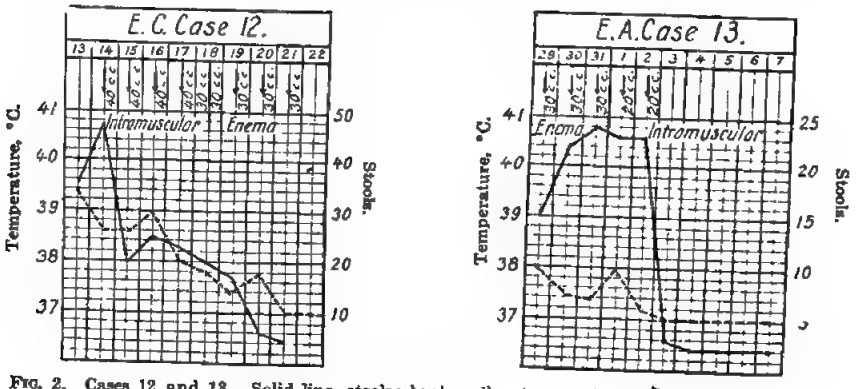

FIG. 2. Cases 12 and 13. Solid line, stools; broken line temperature. One square is equivalent to one stool.

the serum is given, the patient, formerly restless, becomes quiet, the temperature gradually falls, the colicky pains and tenesmus are less severe, and the stools are diminished in number. These results may be observed after from twenty-four to forty-eight hours. In case of callapse, which is present frequently in severe cases, the pulse becomes fuller and stronger a few hours after injection. There is marked abatement of subjective symptoms on the following days. In my experience it seems that the combination of intramuscular administration and serum enemata in acute cases is advisable, for we combata the disease from two sides, namely, (a) neutralization of the toxins in the blood and (b) direct action of the serum on the bacteria and their poisonous products in the lower part of the large intestine. The response of the different patients to administration of serum is shown in fig. 1 (case 8) and in fig. 2 (cases 12 and 13).

The action of the serum introduced intravenously is similar to that of the intramuscular administration, but the effects are more rapid, as shown in fig. 1 (case 18). In the three cases that I treated intravenously with serum, one case was of one day's duration before treatment, and the results are seen in fig. 1 (case 18). In the other two cases, in which the duration of the disease was ten days before treatment, the effect of the serum was not so rapid as in the early case; the temperature fell, and the abdominal pain and tenesmus were diminished, but the frequency of bowel movements was only slightly affected. The reaction following the intravenous injection is mild. Out of three cases one felt a slight chilly sensation beginning forty minutes after injection and lasting only for ten minutes.

The intravenous injection should be made with caution. It is not improbable that serum may produce embolism under certain conditions.

So far as it was possible to gather from the available statistics on the rate of the mortality in bacillary dysentery treated with drugs as compared with the mortality in cases treated with serum, the following data show the observations of other investigators.

a. Cases treated with drugs:
Philippine General Hospital,(5) fiscal year 1912-1913—
Males, 191 with mortality of 17.8 per cent.
Females, 75 with mortality of 20 per cent.
Other hospitals(5)—
Japan, 16.5 to 30.2 per cent mortality.
Singapore (1902), 25.4 per cent mortality.
Ceylon (1903), 28.7 per cent mortality.

Hongkong (1902), 37.3 per cent mortality.
British New Guinea:
1902, 22.80 per cent mortality.
1903, 26.6 per cent mortality.
Egypt, 70 per cent mortality.(7)

b. Cases treated with serum:
Bahr (Fiji Island), 106 cases, 1.8 per cent mortality.(1)
Sandwith (England), 9 per cent mortality.(6)
Shiga (Japan), 9 per cent mortality.(4)
Willmore (Egypt) (1912–1913), 12 per cent mortality.(7)
My cases 20 (1 death), 5 per cent mortality.

In the above figures there is seen a considerable difference in mortality between the two methods of treatment. Undoubtedly the serum has done a great deal toward reducing the death rate.

While my investigations along this line have barely started, and my opportunities have so far been limited, I abstain from drawing definite conclusions for the present, although it cannot be doubted that the value of the specific treatment is strongly indicated as shown in the results hitherto attained. I hope, however, to carry out this work on a larger scale when ample opportunities are presented to me, from which definite conclusions may be drawn.

In conclusion I wish to express my obligations to Professor Ariston Bautista, chief of the Department of Medicine, for allowing me to carry out this work in his department; to Doctors Guerrero, Sison, Domingo, and Gutierrez for valuable suggestions during the course of treatment, and to Doctors Esquivel, Bañuelos, Hilario, Baltazar, and Concepcion for their coöperation. I am especially indebted to Dr. Otto Schöbl, of the Bureau of Science, who furnished me the serum and who made several bacteriological examinations of the stools for me. Also I have to thank him for his kindness in translating the German literature.

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ILLUSTRATIONS

NOTE ON THE PORTAL OF ENTRY IN EXPERIMENTAL CHRONIC PULMONARY (SYSTEMIC) BLASTOMYCOSIS [1]

By H. W. WADE

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That the usual portal of entry in systemic infections by the so-called *Blastomyces dermatitidis* of Gilchrist and Stokes is the respiratory tract itself has been held by most authors who have discussed the matter. This opinion has seemed justified by the usual absence of cutaneous foci or at least of any but apparently secondary lesions markedly in contrast with the usually extensive pulmonary involvement, evidently of long standing. In surprisingly few cases has a distinct history of cutaneous blastomycosis preceding the general invasion been obtained.

The possibility of error in a decision as to the point of origin in a generalized case, based on the evidence of lesions existing late in the disease, has been demonstrated by the findings in monkeys inoculated with this organism. In most of those that have died, especially when the inoculation was peripheral (subcutaneous or intramuscular), pulmonary lesions were found. In all but one animal these were essentially acute, appearing as granulomatous nodules or at the most as small circumscribed abscesses.

In one animal, in which death did not ensue until after a much longer period than usual, lesions developed that resembled those usually found at autopsy in human cases.

This monkey (No. 119), a full-grown, vigorous female, was inoculated on March 26, 1917, subcutaneously on the right side of the belly with 3 cubic centimeters of vigorous bouillon culture. A blastomycotic abscess developed within a few days at the inoculation site, and the inguinal lymph nodes on the same side became markedly enlarged. After about a month the abscess discharged, following which it retrogressed, so that on May 2 only a small, shallow ulcer remained. At this time it was noticed that the animal was well along in pregnancy. On June 10 she delivered an apparently normal young one. Though thin, the mother appeared to be in good health. The cutaneous lesion had practically disappeared, there being only a slight induration

[1] Received for publication December 18, 1917.

beneath the small scar. About July 1 observation was discontinued, the animal being apparently negative.

She did not gain weight, however, and the young one appeared to be undernourished. After three months more both animals were very thin, though in neither was any definite evidence of disease detected. In spite of special care, the mother continued slowly to lose weight and strength and on October 28 was no longer able to sit up. She died that night, seven months after inoculation and four and a half months after delivery. No cough was ever noted, there was no hemorrhage, and as is usual in blastomycosis in the monkey, there was no elevation of temperature on the few occasions when this was taken.

Autopsy. (*October 29, 1917*).—Autopsy disclosed a chronic pulmonary blastomycosis with cavitation very similar to that seen in man. The lobes of both lungs were adherent to each other, and lesions in adjacent lobes had become continuous in places. The visceral and parietal pleuræ were irregularly adherent, chiefly in the upper part, though the blastomycosis itself had not extended to them. The cavities were practically of equal extent in both lungs, involving considerable areas and communicating with the bronchi. They were lined with gray necrotic material of unpleasant odor. The walls were of variable thickness, fibrosed in places, but for the most part actively granulomatous.

The other organs presented no perceptible blastomycotic focus. The liver was somewhat fatty, the spleen was a trifle enlarged and slightly congested, and the kidneys showed a fairly marked cloudy swelling. One small inguinal lymph node on the right side, no larger than a small pea, contained a single drop of pus.

Material from the lungs, in fresh and stained smears and in tissue sections, showed abundant organisms, together with the tissue changes typical of this infection. Cultures were not made, there being no possible doubt as to the identity of the organism. In fresh preparations of material from a cut surface of the spleen occasional blastomycetes were found, though there was no indication of local activity on their part. Preparations from other abdominal viscera were negative for the organisms. A few were found in the pus from the right inguinal lymph node.

DISCUSSION

The lesion described was the only instance of chronic pulmonary infection, similar to that found in human cases, that has developed in a total of about 40 monkeys variously inoculated with this organism. In all other instances that have proved

fatal and in which pulmonary lesions were found these were comparatively acute and were either small granulomatous nodules or small circumscribed abscesses. Metastasis to the lungs has been almost constant and often very extensive, particularly in the monkeys inoculated peripherally; metastases in other viscera have, on the contrary, been comparatively uncommon. In a few cases it has happened, in animals that have been subjected to unfavorable influences, that the focus at the point of subcutaneous inoculation proved comparatively insignificant, in contrast to the extensive pulmonary involvement.

In the animal under discussion pulmonary metastases from the original subcutaneous focus developed, possibly after a period of quiescence, in spite of the subsidence of the peripheral lesion. Whether the metastases would have progressed to a fatal issue in an ordinary animal without the physiological responsibilities of maternity cannot be said, though it seems to me likely that, in spite of the fact that the monkey is more susceptible to this infection than any other laboratory animal, the secondary foci might have been overcome as was the subcutaneous lesion.

The factors that may have influenced the development are for the present discussion incidental to the fact. The point is that had the animal been seen first after the subsidence of the lesion at the point of inoculation, where only a clean, inconspicuous scar remained, there would obviously have been little possibility of recognizing that as the portal of entry of the infection.

It is evident that should similar pulmonary metastasis from a primary subcutaneous or other focus occur in a human case, with subsequent disappearance of the original peripheral lesion, which need never have been particularly large or striking, the onset of the systemic disease might well appear to coincide with or to follow a cold or other temporary disturbance, as, for example, was the history in the majority of the cases reviewed by Stober.[2] This would serve to concentrate attention unduly upon the respiratory tract as the point of origin. In view of the long latency possible in this infection this exacerbation might, as in pulmonary tuberculosis, be at a date comparatively remote from the time of primary invasion.

SUMMARY

A full-grown monkey, later found to be pregnant, was inoculated subcutaneously with the so-called *Blastomyces dermatitidis*, a small abscess resulting. After two and a half months,

[2] Stober, A, M., *Arch. Int. Med.* (1914), **13**, 509.

at which time the abscess had healed, she delivered a healthy young one. Neither thrived; the mother slowly became thinner and died of exhaustion four and one-half months after delivery. Autopsy revealed a bilateral, chronic pulmonary blastomycosis similar to that usually found in human cases. From the evidence presented during the last five months of life, or elicited by autopsy, the erroneous conclusion that the infection was primarily pulmonary could hardly have been avoided. By analogy it seems possible for systemic blastomycosis in man to be similarly inaugurated, perhaps at a considerable time before, by a temporary or inconspicuous peripheral lesion. Therefore, though it is highly probable that the respiratory tract may more or less frequently be primarily affected, the conclusion that this is the constant or even the usual portal of entry is hardly to be justified on the basis of imperfect history or of late clinical and autopsy findings.

PRESERVATION OF CHOLERA STOOL SPECIMENS FOR DELAYED BACTERIOLOGIC EXAMINATION [1]

By C. S. PANGANIBAN and OTTO SCHÖBL

(From the Serum Section of the Biological Laboratory, Bureau of Science, Manila)

The importance of bacteriologic diagnosis of Asiatic cholera is generally appreciated by practical sanitarians. Its importance increased considerably since it became generally known that by means of bacteriological examination of fæces the detection of cholera carriers could be made possible. As reliable as the laboratory procedure is to detect the cholera vibrios when present in the fæces even in small numbers, the rapid disappearance of cholera vibrios in the fæces exposed to higher temperature particularly in presence of other intestinal bacteria is no doubt responsible for the failures in delayed examinations. It would, therefore, be of some value if a method could be devised to preserve the specimen for such a length of time as is necessary to transport it from the source to a laboratory equipped for bacteriological diagnosis of cholera.

Several ways suggest themselves from previous experiences of others and our own.

1. The method of preserving fæces with glycerin suggested by Teague [2] for delayed examination of typhoid stools.

2. Sodium chloride solution. It was found in previous researches concerning the survival of cholera vibrios in water that cholera vibrios remain alive in sea water for a considerable length of time—in our particular experiment, between 106 and 120 days.[3] This experience could be probably made use of.

3. Bile. This medium, first recommended by Otolenghi, possesses selective properties of high degree with regard to cholera vibrios. Cholera vibrios in pure culture survive in bile for an indefinite length of time. This fact was referred to briefly in a previous communication [4] and has been utilized

[1] Received for publication February 21, 1918.

[2] Teague, Oscar, and Clurman, A. W., *Journ Inf. Dis.* (1916), **18**.

[3] Schöbl, Otto, *Phil. Journ. Sci., Sec. B.* (1914), **9**, 479.

[4] Idem, *Journ. Inf. Dis.* (1916), **18**, 307.

since by keeping stock cultures of cholera growing in bile medium.

The present investigation consists of three experiments, namely, with glycerin, with sodium chloride solutions, and with bile, respectively. Although these experiments have not been carried out simultaneously, the salt solution used in each of the experiments serves as *tertium comparationis* and allows fair comparison of the results of the three groups of experiments.

EXPERIMENT I. GLYCERIN

About 3 grams of stools were ground up in 30 cubic centimeters of 0.6 per cent salt solution. The mixture was filtered through cheesecloth. Twenty cubic centimeters of 0.6 per cent salt solution were added to two 24-hour old cultures of cholera vibrios, the cultures were washed, and the suspension was added to the filtrate of the stool mentioned above. Thus prepared artificial cholera-stool emulsion was thoroughly shaken in a flask containing glass beads.

Five cubic centimeters of 0.6 per cent salt solution and 40 per cent, 50 per cent, and 60 per cent glycerin, respectively, were put into four separate sterile test tubes, and to each one of these were added 5 cubic centimeters of the above suspension of fæces. The tubes were then thoroughly shaken and left at room temperature (32° C.). One peptone water culture was planted from each of the four tubes immediately by transferring three loopfuls of their content. After twenty-four hours' incubation Dieudonné's plates were made from the peptone cultures. At the end of twenty-four hours' incubation the colonies that developed on the plates were examined. Smears were made, and microscopic agglutination was performed.

The three tubes containing the mixture of artificial cholera fæces and glycerin in various strength of percentage as well as the fourth tube containing no glycerin but only salt solution were examined daily for the presence or the absence of cholera vibrios by the procedure just described.

The results of this experiment (Table I) show that glycerin has no preserving action for cholera vibrios. In 20 per cent and in 25 per cent concentrations the cholera vibrios survived four days only, while in the tube containing 30 per cent glycerin no cholera vibrios could be found on the fourth and the subsequent days. These findings are in accord with those obtained by Ruediger, who made similar observations while testing the germicidal action of glycerin.

EXPERIMENTS II AND III. SALT SOLUTION AND OX BILE

Five cubic centimeters of sterile sodium chloride solution, in concentration of 0.5 per cent, 1 per cent, 2 per cent, 5 per cent, 10 per cent, 15 per cent, 20 per cent, 25 per cent, and 30 per cent, were placed into a set of sterile test tubes, and 5 cubic centimeters of 50 per cent, 75 per cent, and pure ox bile were put into the tubes of the second set. In both of these sets one tube containing 5 cubic centimeters of sterile normal salt solution was used as a control.

About 10 grams of normal stool were ground up in 100 cubic centimeters of sterile normal salt solution, and the mixture was filtered through cheesecloth. Ten cubic centimeters of sterile normal salt solution were added to each of four 24-hour-old agar cultures of cholera vibrio, which were then washed, and the suspension was added to the filtered emulsion of the stool. The final mixture of cholera vibrios and stool was thoroughly shaken in flasks containing glass beads. Five cubic centimeters of this suspension were then added to each one of the tubes containing varying concentrations of salt solution and ox bile, respectively. The tubes, after the addition of cholera stool suspension, were thoroughly shaken and were kept at room temperature (32° C.). Immediately after mixing, one peptone water culture was planted from each of the tubes by transferring three loopfuls of the stool emulsion. After twenty-four hours' incubation Dieudonné's plates were planted from the peptone culture. At the end of another twenty-four hours' incubation the colonies that developed were examined microscopically. Smears were made, and miscroscopic agglutination was performed.

Similarly peptone water cultures, Dieudonné's plate, and microscopic examinations of the colonies were made every day during the first week, after which time examinations were made once a week only. It is evident from Table II that sodium chloride preserved the cholera fæces successfully for the period of five weeks, at least, in concentration of from 0.5 to 5 per cent. In concentration higher than 5 per cent the vibrios could not be found after five and four days, respectively.

In Table III the results of the experiment, in which bile was used as preservative, are tabulated. It shows successful preservation of cholera fæces in dilution of 50 per cent, 75 per cent, and 100 per cent of bile with normal salt solution as a control.

In the previous experiments the sodium chloride solution and ox bile gave equally good results as preservatives of cholera stools for delayed examination when the amount of cholera

vibrios in the fæces was considerable, but when the amount of cholera vibrios in the fæces was decreased, the ox bile proved to be superior to the sodium chloride solution (Tables IV and V).

CONCLUSIONS

1. Glycerin, sodium chloride solution, and ox bile were tested for their value as preservatives of cholera fæces for delayed bacteriological examination.

2. Glycerin was found unfit for that purpose, while sodium chloride solution and bile gave good results.

3. Ox bile was found superior to sodium chloride solution in those instances where the cholera vibrios were present in the fæces in small numbers.

TABLE I.—*Showing the preserving action of glycerin on cholera vibrios.*

PLATE FROM PEPTONE.

	Glycerin.			Sodium chloride.
	20 per cent.	25 per cent.	30 per cent.	
Immediately	+	+	+	+
24 hours	+	+	+	+
48 hours	+	+	+	+
72 hours	+	+	+	+
Fourth day	+	−	−	+
Fifth day	−	−	−	+
Sixth day	−	−	−	+

TABLE II.—*Showing the preserving action of salt solution on cholera vibrios.*

Concentration of salt solution.	Dieudonné's plate.											
	In days.								In weeks.			
	0	1	2	3	4	5	6	7	2	3	4	5
0.5 per cent	+	+	+	+	+	+	+	+	+	+	+	+
1 per cent	+	+	+	+	+	+	+	+	+	+	+	+
2 per cent	+	+	+	+	+	+	+	+	+	+	+	+
5 per cent	+	+	+	+	+	+	+	+	+	+	+	+
10 per cent	+	+	+	+	+	−	−	−	−	−	−	−
15 per cent	+	+	+	+	+	−	−	−	−	−	−	−
20 per cent	+	+	+	+	−	−	−	−	−	−	−	−
25 per cent	+	+	+	+	−	−	−	−	−	−	−	−
30 per cent	+	+	+	+	−	−	−	−	−	−	−	−
Control (normal salt solution)	+	+	+	+	+	+	+	+	+	+	+	+

TABLE III.—*Showing the preserving action of ox bile on cholera vibrios.*

DIEUDONNÉ'S PLATE.

Concentration of bile in H_2O.	In days.								In weeks.			
	0	1	2	3	4	5	6	7	2	3	4	5
50 per cent	+	+	+	+	+	+	+	+	+	+	+	+
75 per cent	+	+	+	+	+	+	+	+	+	+	+	+
Pure bile	+	+	+	+	+	+	+	+	+	+	+	+
Control (normal salt solution)	+	+	+	+	+	+	+	+	+	+	+	+

TABLE IV.—*Showing the preserving action of 1 per cent salt solution on cholera vibrios.*

DIEUDONNÉ'S PLATE.

Amount of cholera vibrios in terms of loop.	In days.								In weeks.					
	0	1	2	3	4	5	6	7	2	3	4	5	6	7
2	+	+	+	[a]										
1	+	+	+											
0.5	+	+	+											
0.1	+	+	+											
0.01	+	+	+											
0.005	+	+	+											
0.001	+	+	+											
0.0005	+	+	+											
0.0001	+	+	+											
0.00005	+	+	+											
0.00001	+	+	+											
0.000005	+	+	+											
0.000001	−	+	+	+	+	+	+	+	+	+	[b]			
0.0000001	−	−	+	+	+	+	+	+	+	+				
0.00000005	−	−	+	+	+	+	+	+	+	+				
0.00000001	−	+	+	+	+	+	+	+	+	+				
0.000000005	−	−	+	+	+	+	+	+	+	+	+	+	+	−
0.000000001	−	−	+	+	+	+	+	+	+	+	+	+	+	−
0.0000000005	−	−	+	+	+	+	+	+	−	+	−	−	−	−
0.0000000001	−	−	−	+	+	+	+	+	−	+	−	−	−	−

[a] Not tested after two days.

[b] Not tested after three weeks.

TABLE V.—*Showing the preserving action of pure ox bile on cholera vibrios.*

DIEUDONNÉ'S PLATE.

Amount of cholera vibrios in terms of loop.	In days.								In weeks.					
	0	1	2	3	4	5	6	7	2	3	4	5	6	7
2	+	+	+	[a]										
1	−	−	−											
0.5	+	+	+											
0.1	+	+	+											
0.01	+	+	−											
0.005	+	+	+											
0.001	+	+	−											
0.0005	+	+	−											
0.0001	+	+	+											
0.00005	+	+	+											
0.00001	+	+	+											
0.000005	−	+	+											
0.000001	+	+	+	+	+	−	−	+	+	+	[b]			
0.0000001	−	+	+	+	+	+	+	+	+	+				
0.00000005	−	+	+	+	+	+	+	+	+	−				
0.00000001	−	+	−	+	−	+	+	+	+	+				
0.000000005	−	+	+	+	+	+	+	+	+	+	+	+	+	+
0.000000001	−	+	+	+	+	+	+	+	+	+	+	+	+	+
0.0000000005	−	+	−	+	+	+	+	+	+	+	+	+	+	−
0.0000000001	−	+	+	+	+	−	+	+	+	+	+	+	+	+

[a] Not tested after two days.

[b] Not tested after three weeks.

REVIEWS

A Practical Text-book | of | Infection, Immunity | and Specific Therapy | with special reference to immunologic technic | by | John A. Kolmer, M. D., Dr. P. H., M. Sc. | [4 lines] | with an introduction by | Allen J. Smith, M. D., Sc. D., LL. D. | [1 line] | with 147 original illustrations, 46 in colors | by Erwin F. Faber | [1 line] | second edition, thoroughly revised | Philadelphia and London | W. B. Saunders Company | 1917. Cloth, pp. i–xiii—1–978. Price $7.00 net. Half morocco, $8.50.

The following is from the preface to the second edition:

Additions and alterations have been made throughout; special attention has been given the subject of local infection; the Schick toxin test for immunity in diphtheria and active immunization in diphtheria and active immunization in diphtheria with toxin-antitoxin mixtures; complement-fixation in tuberculosis and other bacterial infections and a quantitative Wassermann reaction based upon my studies with the co-operation and assistance of Dr. Claude P. Brown, Dr. Toitsu Matsunami, and Dr. Berta Meine, aiming to standardize this important test. The chapters on anaphylaxis have been revised and particular attention given the subject of anaphylactic skin reactions. Lange's colloidal gold reaction has been included. The chapter on the treatment of various infections with bacterial vaccines has been enlarged and the non-specific activity of bacterial vaccines discussed. The section on the treatment of certain of the acute infectious diseases, and particularly acute anterior poliomyelitis, with the serum of convalescents and normal persons has been amplified; blood transfusion has been included. Special attention has been devoted to the chapter on Chemotherapy, and the results of the studies of Dr. Jay F. Schamberg, Dr. George Raiziss, and the author bearing upon the toxicity of salvarsan and its congeners and the reactions following their administration have been included and discussed. The subject of Bacterial Chemotherapy, which promises much in the future, has been amplified from the theoretical and technical viewpoints.

American Addresses | by | Sir Berkeley Moynihan, M. S., F. R. C. S. | [ornament] | Philadelphia and London | W. B. Saunders Company | 1917. Cloth, pp. 1-143. Price $1.75.

From the preface we quote the following:

The papers included in this volume were read in Chicago and elsewhere during October and November, 1917. I hope they may help my American colleagues to some appreciation of the causes and conditions of the war, and afford some help to them in their treatment of the many phases of surgical diseases with which they will be called upon to deal.

The papers represent not only my own views and experience, but those of others also. In forming my opinions upon the several matters dis-

cussed I have received very great help from the many consultations and discussions I have had with many of my friends in the different war zones of France and England.

The book contains the following chapters: The causes of the war; gunshot wounds and their treatment; wounds of the knee-joint; on injuries to the peripheral nerves and their treatment; and gunshot wounds of the lungs and pleura.

Tumors of the Nervus Acusticus | and the Syndrome of the Cerebellopontile Angle | By | Harvey Cushing, M. D. | [five lines] | Illustrated | Philadelphia and London | W. B. Saunders Company | 1917. Pp. i–viii—1–296. Cloth, $5.00 net.

From the preface we quote the following:

In the course of preparation of a monograph dealing with a series of meningeal fibro-endotheliomata, a careful review was necessitated of the pathological as well as the clinical aspects of these interesting tumors. They have their point of origin in certain definite regions, and a tentative subdivision had been made of those arising from the spinal meninges, those from the basilar meninges, and those from the superior envelopes of the brain.

It was apparent that the spinal and basilar lesions usually arose from the meninges at the point of exit of a spinal or cerebral nerve root, and it was anticipated that many of the tumors of the cerebellopontile angle which involve the acoustic nerve would be included in the series, for the majority of them had previously been diagnosed from their gross appearance, though admittedly with some reservation on histological grounds, as endotheliomata.

Hesitation was felt in regard to the inclusion in the series of some of the spinal cord tumors, and these doubts became intensified when the lateral recess tumors came to be assembled and closely inspected.

A thorough rehearsal of the material at hand, comprising twenty-nine histologically certified cases, together with a much larger number of probable though unverified ones, which nevertheless were useful from the standpoint of their clinical data, so clarified many obscure matters relating to these peculiar and unmistakable tumors of the VIIIth nerve that they have been made the subject of this separate study, and a report upon the 60 endotheliomata proper must await its turn. Unquestionably the acoustic tumors are most distinctive growths and such relationship as they have to the meningeal tumors occurring in the lateral recess will be pointed out in its proper place.

Some important monographs on the subject have already been published, of which Folke Henschen's Inaugural Dissertation, 1910, is the most noteworthy, but in all of them the various tumors of the cerebellopontile angle have been incorporated, whereas the acoustic neuromas will alone occupy our attention to the exclusion of other tumors of the recess except in so far as they are of interest from the standpoint of differential diagnosis.

Issued with THE PHILIPPINE JOURNAL OF SCIENCE, SECTION B, Tropical Medicine, Vol. XIII, No. 5, September, 1918.

PROCEEDINGS OF THE MANILA MEDICAL SOCIETY

REGULAR MONTHLY MEETING, APRIL 1, 1918

MINUTES OF THE MANILA MEDICAL SOCIETY

The regular meeting of the Manila Medical Society was held at the College of Medicine and Surgery, April 1, 1918, at 9 in the evening.

One visitor and seven members were present.

The minutes of the previous meeting were read and approved as read.

On the recommendation of the council, the application of Drs. Herminio Velarde y Esquivel and Walfrido de Leon for active membership was ratified by the society.

In the absence of Dr. J. A. Johnston, his interesting paper on Twenty-five Years of Laboratory Work was read by Dr. R. B. Gibson.

Dr. José S. Hilario followed with a paper entitled the Widal Test at Different Stages of Typhoid Fever, which was discussed by Doctors Gonzales, Vincent, and de la Paz.

Dr. Isabelo Concepción presented a paper on Analysis of Normal Filipino Urine. This paper was discussed by Doctors Gibson, Gonzales, Hilario, and de la Paz.

Doctor Esquivel, visitor, next demonstrated a case of splenomegaly.

The chairman announced that by action of the council the meeting of the society during May, June, and July would be suspended on account of warm weather and the next meeting would be in August.

The meeting was adjourned at 11.15.

D. DE LA PAZ,
Secretary-Treasurer,
Manila Medical Society.

SCIENTIFIC PROGRAM

TWENTY-FIVE YEARS OF LABORATORY WORK

By DR. JOHN A. JOHNSTON

An interesting contrast of the workers, methods, and laboratories of twenty-five years ago with those of to-day.

THE GRUBLER-WIDAL TEST AT DIFFERENT STAGES OF TYPHOID FEVER

By Dr. J. S. Hilario

The diagnosis of infectious diseases by means of agglutination test is one of the most useful means that is available to recognize infectious diseases such as typhoid fever. The present paper gives the findings in 818 cases submitted to the Grubler-Widal test, of which 607 were typhoid, 154 were doubtful or suspected typhoid, and 77 were other diseases. Of 818 tests performed, 404 were positive, giving a percentage of 56.8 positive. The test was performed with *Bacillus typhosus*, *B. paratyphosus-A*, and *B. paratyphosus-B* simultaneously on each individual case. During the first week of typhoid infection the percentage positive found was 42.3; during the second week, 65.4; during the third week, 65.9; during the fourth week, 72.8; during the seventh week, 100. A series of 440 cases submitted to simultaneous tests with three organisms of the typhoid-paratyphoid group has given the following results: With *Bacillus typhosus* the positive tests were 52.04 per cent; with *B. paratyphosus-B*, 30.04 per cent; with *B. paratyphosus-A*, 9.77 per cent. In a series of 226 positive tests the presence of group-agglutinations among members of the typhoid-paratyphoid group is shown by the following: Group-agglutination between *typhosus* and *paratyphosus-B* was found in 44.6 per cent; between *typhosus* and *paratyphosus-A* in 5.3 per cent; and between *typhosus*, *paratyphosus-A* and *paratyphosus-B* in 6.6 per cent. As to diseases other than typhoid the results of the test made were as follows: Of 103 cases of undetermined fever 43 were positive for *typhosus*, giving an average of 41 per cent; of 4 cases of malaria, 1 was positive for *typhosus* and 1 for *paratyphosus-B;* of 3 cases of pneumonia, 1 was positive for *typhosus;* of 2 cases of meningitis, 1 was positive for *typhosus;* of 1 case of pulmonary tuberculosis, 1 was positive for *typhosus;* of 2 cases of bronchopneumonia, both were positive for *typhosus;* and 1 case of dysentery and 1 case of puerperal fever were also positive for *typhosus*. In view of the above data, the following conclusions may be established:

(1) Infection with *Bacillus typhosus* is the most frequent in Manila, while paratyphoid infections also occur, although not so frequently, the infection with *paratyphosus-B* being more frequent than that with *paratyphosus-A*, which is rare.

(2) Typhoid-paratyphoid-B group-agglutinations are the com-

monest, representing about 50 per cent of all the group-agglutinations observed.

ANALYSIS OF NORMAL FILIPINO URINE

By Dr. Isabelo Concepción

Analyses were reported of the twenty-four hour urines of students, laboratory helpers, prisoners, and hospital servants. Characteristic findings for Filipinos are a small volume, low total nitrogen (3.05 to 12.63 grams), a low percentage of urea nitrogen with reference to the total nitrogen, low uric acid (average 0.376 gram), and low chlorides (average 5.86 grams as sodium chloride). The ammonia, the creatinine, the undetermined nitrogen, and the ratios nitrogen phosphoric acid and nitrogen sulphur are in accord with the low nitrogen values of the Filipino dietary.

R. B. Gibson,
Editor of the Proceedings,
Manila Medical Society.

The Philippine Islands Medical Association held a joint scientific and social session February 4 to 9, inclusive, with the IV Asamblea Regional de Medicos y Farmaceuticos de Filipinas. The scientific proceedings will be published with those of that organization. At the close of the scientific sessions, the business meeting of the association was adjourned to follow some meeting of the Manila Medical Society in the near future at the call of the secretary-treasurer, at which time the election of officers was to be held.

MINUTES OF THE PHILIPPINE ISLANDS MEDICAL ASSOCIATION

Postponed business meeting, April 1, 1918.

The meeting was called to order at the close of the regular monthly meeting of the Manila Medical Society, the vice-president, Doctor Calderon, presiding.

The report of the nominating committee was presented and on motion, duly seconded, the secretary-treasurer was instructed to cast the ballot for the association for the following nominees:

President:	Dr. Fernando Calderon.
Vice-presidents:	Dr. E. S. Ruth.
	Dr. Jesus Gonzalez.
Councilor:	Dr. Daniel de la Paz.

There being no further business, the meeting was adjourned.

R. B. Gibson,
Secretary-Treasurer,
Philippine Islands Medical Association.